IJPSR (2019), Volume 10, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 May 2019; received in revised form, 24 September 2019; accepted, 22 October 2019; published 01 November 2019

ANALYSIS OF TARAXEROL AND TARAXASTEROL IN HAIRY ROOT CULTURES OF *TARAXACUM OFFICINALE* WEBER

Kiran Sharma

Department of Pharmacognosy and Phytochemistry, SGT College of Pharmacy, Gurugram - 122505, Haryana, India.

Keywords:

Taraxacum officinale (TO), Hairy roots, *Agrobacterium rhizogenes*, Taraxerol, Taraxasterol, RP-HPLC

Correspondence to Author:
Dr. Kiran Sharma

Assistant Professor (Pharmacognosy), SGT College of Pharmacy, Gurugram - 122505, Haryana, India.

E-mail: kiransharmapharma@gmail.com

ABSTRACT: *Taraxacum officinale* (TO) Weber commonly known as dandelion has contained triterepenes-taraxasterol and taraxerol in its roots which are nowadays being recognized as potent anticancer compounds. The main objective behind the present study was to increase the yield of taraxasterol and taraxerol through induction of hairy roots in *Taraxacum officinale* Weber using two strains of *Agrobacterium rhizogenes* MTCC-532 and MTCC-2084. An efficient protocol for the induction of hairy roots is proposed from the root callus cultures of *Taraxacum officinale* maintained on MS + IAA + BA + 2, 4-D (0.5 ppm + 1 ppm + 0.5 ppm) through infection of the root callus with two strains of *Agrobacterium rhizogenes* MTCC-532 and MTCC-2084. It was found that MTCC-532 proved to be effective in hairy roots induction whereas MTCC-2084 failed to induce hairy roots. HPLC results showed the retention time of taraxerol and taraxasterol at 4.975 and 3.5 min respectively. Showing fourfold increase in content of taraxasterol and a nearly two-fold increase in taraxerol.

INTRODUCTION: *Taraxacum officinale* (TO), a traditional medicinal herb that belongs to family Asteraceae, popularly known as common dandelion has been used in herbal medicines as reported by bajaj in 1994, for it's anti-inflammatory, anti-rheumatic, diuretic, chloretic, and anti-carcinogenic properties. Roots of TO are known to possess hypolipidemic, anti-carcinogenic, anti-ulcer, anti-oxidant, anti-viral and prebiotic activities. Also the roots are used as substitute for caffeine-free coffee and as a herbal tea. Its leaves are used as salad and anti-rheumatic activities are shown by aerial parts.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(3).5094-02

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(3).5094-02

Whole plants possess antiallergic, anticoagulatory, anti-hyperglycemic, analgesic properties and flowers are used for making wine. Dandelion roots contain various types of pentacyclic triterpenoids (*e.g.*, ursane, oleanane, and taraxastane types) of which taraxasterol and taraxerol are the important compounds that possess important pharmacological activities. Also taraxerol has shown therapeutic activity in Alzheimer's and Parkinsonism.

Taraxerol has been used to induce apoptosis, acetylcholinesterase inhibitor, COX inhibitor and possess anti-microbial potential. Taraxasterol is anti-allergic, anti-oxidant and anti-inflammatory and anti-carcinogenic. Also these compounds are known for their activity in Alzheimer's. The occurrence of TX and TA has been reported in nature by Sharma & Zafar [1]. The genus Taraxacum has a bit complicated taxonomy by polyploidy and apomictic lineages.

Further complications of the taxonomy of this species in past occurred as result of microspecies, subspecies, and numerous species recognition. For the species of Taraxacum, more than 2500 specific epithets have been described [2]. So due to large amount of biodiversity, there may be variations in the amount of phytoconstituents and hence it may be advisable to analyze many plant samples from different geographical locations. Plant biotechnology / plant tissue culture represents a promising technology for the production of medicinal plants and their active constituents and also a convenient experimental system for plant science research. Both taraxerol and taraxasterol acquire high therapeutic efficiency, and hairy root culture proves to be a promising technology for improving the yield of these secondary metabolites because hairy roots are source of continuous, stable and higher yield of secondary metabolites.

Agrobacterium rhizogenes when infects a plant part or plant, a root inducing (Ri) plasmid (where DNA is present in the form of a small ring) is transferred to the plant cell. Hence, the infected plant cell starts behaving like a root cell and thus numerous, fast-growing hairy roots are produced as result of the infection. Consequently, an alteration in the production of auxin is also seen, a plant growth hormone that is responsible for the fast-growing adventitious roots proliferation. Genes for unusual amino acid called opines are also carried by the Ri plasmid. Transformation is confirmed by the production of opines [3].

Using *A. rhizogenes* the first directed trans-formation of higher plants was made by Ackermann and later it proved to be one of the most promising techniques of production of secondary metabolites [4, 5]. The primary objective of the current study was to establish a stable hairy root culture and quantify the presence of taraxasterol and taraxerol in it.

MATERIAL AND METHODS:

Plant Material: *Taraxacum officinale* (TO) was grown in herbal garden Jamia Hamdard, New Delhi after bringing it from Kashmir and collected from the herbal garden. The authentification of the plant and seeds were done by Dr. Sunita Garg at NISCAIR (Raw Materials Herbarium and Museum), Pusa Campus, Dr. K. S. Krishnan Marg, New Delhi- 110012 and its assigned voucher specimen number was NISCAIR/RHMD/Consult/ 2013/2273/53.

Agrobacterium-Mediated Hairy Root Culture: The explants used for hairy root culture initiation was root callus and the leaf of TO.

(i) In order to initiate hairy root culture on roots of TO, root callus experiment was performed. The seeds were collected in the month of October and washed 2-3 times with water, again washed with teepol solution and rinsed with tap water 2-3 times thoroughly and then treated for 6 min with 0.1% sodium hypochlorite. These sterile seeds were then transferred aseptically into sterile petriplates containing filter paper and absorbent cotton. The petriplates were then covered with aluminum foil and kept in BOD incubator at conditions (24 ± 2 °C, 55-60% RH) for germination. Seeds showed germination after one week. The root tip of the aseptically grown seedlings was cut and transferred aseptically into solid Murashige and Skoog's (MS) medium as basal medium containing the same hormone combination. The callus initiation was seen after 15 days. After initiation of root callus, the calli were aseptically separated from the mother explants and the calli initiated on root in 15 days were transferred to the MS medium containing the same hormone combinations in order to develop an independent calli. The growth was observed for further 3 weeks. The independently developed calli were chopped down and transferred aseptically in MS medium supplemented with a similar hormonal combination for the initiation of hairy root culture.

(ii) In order to initiate hairy root culture from leaves of TO, the leaves were collected from natural plant growing in herbal garden of Jamia Hamdard, in the month of June. Leaves were then repeatedly washed with water 2-3 times, teepol solution, then again washed with water 2-3 times, then washed with double distilled water 2-3 times and then sterilized for 5 min with 0.1% $HgCl_2$. After that leaves were washed repeatedly 2-3 times with autoclaved double distilled water. Then by means of aseptic blade cuts were made on the sterilized leaves and they were then inoculated aseptically into culture tubes containing solid MS medium with hormone combinations (MS + IAA + 2, 4-D 1 ppm each) in laminar airflow. After that

the culture tubes were kept in BOD (24 ± 2 °C, 55-60% RH) for callus initiation.

Bacterial Strains and Cultures: Two strains of *Agrobacterium rhizogenes* MTCC-532 and MTCC-2084 were obtained from Microbial Type Culture Collection and Gene bank, Institute of microbial technology, sector-39 A, Chandigarh, India and stored in the freezer. The glass tubes in which the strains were packed aseptically in the form of lyophilized powder were broken and both the strains were streaked on LB solid agar plates with the help of aseptic loop and both the petriplates of strains MTCC-532 and MTCC-2084 were incubated at 25 °C for 24 h and 48 h respectively to see the appearance of colonies **Fig. 1** and inoculation of a single bacterial colony of each strain was done into 10 ml of liquid LB medium to maintain their pure stock culture.

The optical density of the pure stock cultures of each strain was adjusted to 0.5-0.7 OD at 600 nm. Further various dilutions from the bacterial stock solution were prepared and the dilution in conc. of 1:109 showed countable colonies of bacteria and it was used for the infection of the explant with the *Agrobacterium rhizogenes* strains and genetic transformation.

FIG. 1: COLONIES OF AGROBACTERIUM RHIZOGENES-MTCC-532

Establishment of Transformed Hairy Roots: (i) The infection of the root callus explants with countable colonies of dilution of *Agrobacterium rhizogenes* strain was done in the following ways.

- In the first method, infection of the root callus by *Agrobacterium rhizogenes* solution was done by simply pouring the bacterial solution over the surface of the root callus.
- In other methods, pricking of the root callus was done (shown in **Fig. 2**) with the help of a sterile scalpel and addition of the bacterial solution was done without disturbing/ disorienting the callus cells.
- In the third method addition of the bacterial solution was done somewhat forcefully leading to the disorientation of the root callus cells.

FIG. 2: ROOT CALLUS MAINTAINED ON MS + IAA + BA + 2, 4-D (0.5 PPM + 1 PPM + 0.5 PPM), INFECTED WITH *A. RHIZOGENES* STRAINS DILUTIONS SHOWING COUNTABLE COLONIES AFTER PRICKING THEM ASEPTICALLY WITH NEEDLE AND INOCULATING THEM ON SOLID MS MEDIUM FOR 24 h WITH THE HORMONE COMBINATIONS MENTIONED ABOVE

After that using sterile Whatman no.1 filter paper, the infected root callus were blotted dry and co-cultivated on solid MS medium with similar hormone combination required for root callus initiation at 24 ± 2 ºC for 48 h in dark. After the co-cultivation process, explants were removed and washed with sterile double distilled water 4-5 times. Again they were blotted dry with the help of sterile Whatman filter paper no. 1 and transferred to MS medium containing 250 µg/ml and 500 µg/ml of antibiotic cefotaxime and kept for incubation at 24 ± 2 °C. After the initiation of hairy roots was observed repeated subculturing on fresh medium was done every three weeks and the maintenance of hairy roots was done on solid half strength or full-strength MS medium independent of the hormones.

Antibiotic addition was continued to MS medium until contamination was totally absent. Once hairy root cultures were established showing total absence of bacterial contamination and then the subculturing was done on liquid MS media without

antibiotics and hormones. After every 15 days the growth of hairy roots was observed, and an average of 3 samples was taken for determining the rate of growth by increase in biomass.

(ii) For carrying out the transformation experiment on leaf explants and leaf explants showing callus initiation with hormone combination MS + IAA + 2, 4-D (1 ppm each). The following procedure was followed.

- In the first method, sterilized leaf explants as well as leaf explants showing callus initiation in 15 days were pricked with needle on the midrib and infection with *Agrobacterium rhizogenes* strain was done by shaking the explants and 20 ml of bacterial dilution in 100 ml conical flask on a BOD shaker for a interval of 5, 10, 15, 20 and 25 min.

- In the second method, the bacterial inoculums were directly injected into the midrib of leaf explants.

After that using sterile Whatman no.1 filter paper, the infected leaf explants were blotted dry and co-cultivated on solid MS medium with similar hormone combinations required for root callus initiation at 24 ± 2 °C for 48 h in dark. After the cocultivation process, explants were removed and washed with sterile double distilled water 4-5 times. Again they were blotted dry with the help of sterile Whatman filter paper no-1 and transferred to MS medium containing 250 µg/ml and 500 µg/ml of antibiotic cefotaxime (Cadila Healthcare, Ahmedabad) and kept for incubation at 24 ± 2 °C.

After the initiation of hairy roots was observed repeated subculturing on fresh medium was done every three weeks and the maintenance of hairy roots was done on solid half strength or full-strength MS medium independent of the hormones. Anti-biotic addition was continued to MS medium until contamination was absent.

Once hairy root cultures were established showing total absence of bacterial contamination and then the subculturing was done on liquid MS media without antibiotics and hormones. After every 15 days the growth of hairy roots was observed, and an average of 3 samples was taken for determining the rate of growth by increase in biomass. The protocol for the induction of hairy roots has been detected in **Fig. 3.**

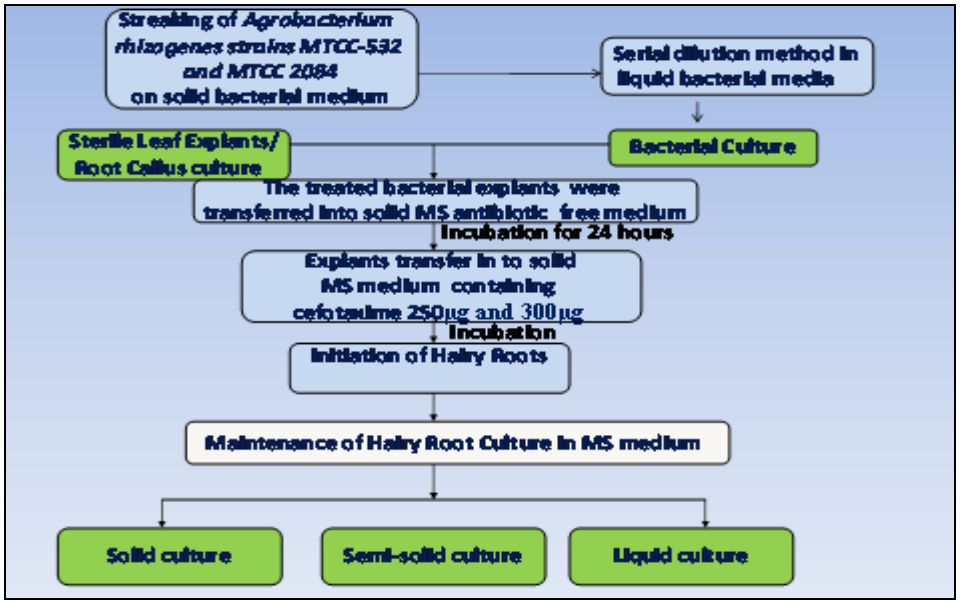

FIG. 3: PROTOCOL FOR INITIATION OF HAIRY ROOT CULTURE

Estimation of Taraxasterol and Taraxerol by HPLC:

Reagents and Chemicals: Water (HPLC grade) was purchased from Loba Chemie (Mumbai, India) and Acetonitrile (HPLC grade) and Methanol (HPLC grade) was purchased from Merck Specialties Pvt. Ltd. (Worli, Mumbai, India) and phosphoric acid AR was purchased from Merck and S. D. Fine Chemicals, Mumbai respectively. Working standards of taraxasterol and taraxerol

were provided by eBiotech Inc., Devali, New Delhi. All other reagents used were of HPLC grade.

Preparation of Standard Solutions: Standard stock solution of taraxasterol and taraxerol 1mg/ml was prepared by dissolving in methanol followed by ultra-sonication.

Extraction of Root Callus and Preparation of Sample Solutions: 1 gm each of powdered natural root, root calli, and hairy root were dried at 60 °C was extracted with 25 ml of methanol separately, filtered and filtrates were concentrated. The final volume was made up to 10 ml. 1 ml solution was taken out from this and filtered through 0.2 µm membrane filter (Gelman Science, India). The samples were analyzed by HPLC for the quantitative estimation of taraxasterol and taraxerol by a Shimadzu model HPLC equipped with quaternary LC-10A VP pump, ODS, C8-3 column (250 mm × 4.6 mm i.d, particle size 5 µ) was used for the separation, variable wavelength programmable UV/VIS detector, SPD-10AVP column oven (Shimadzu), SCL 10AVP system controller (Shimadzu), rheodyne injector fitted with a 20 µl loop. Class-VP 5.032 software was used for the routine drug analysis.

Determination of Content of Taraxerol (TA) and Taraxasterol (TX): The content of TA and TX were determined by the HPLC method reported by Sharma & Zafar [6].

Detection of Opine in Hairy Root Culture of *Taraxacum officinale*: In order to confirm the transgenicity of hairy root cultures, 100 mg 60 days old hairy roots were taken and extraction was done with 1% HCl (20 ml) for 10 min at 100 °C. Then the suspension was subjected to centrifugation and the supernatant layer was evaporated at 40 °C and dissolved in 2 ml water. Extracts (2-10 µl) and standard agropine and manopine were applied on Whatman 3 MM paper and subjected to high voltage electrophoresis in formic acid: acetic acid: water (30:60:910 v/v) at 10 v cm^{-1} (1.9). After drying in hot air, the opines were visualized by spraying with alkaline silver nitrate solution. The R_f value was determined [7].

RESULTS AND DISCUSSION:

Initiation of Root Callus and Leaf Callus: When cultured in solid MS media without growth hormones, the root tip of the sterile plantlets showed slow or no growth. Satisfactory, results were obtained when these sterile rootlets were transferred to the sterilized culture tubes containing MS medium supplemented with different hormonal combinations, but a combination that proved to be the best for initiation and growth of callus were MS + IAA + BA + 2, 4-D (0.5 ppm + 1 ppm + 0.5 ppm). The root callus was initiated with this hormone combination in 20 days and maintained without any contamination. The growth kinetics of callus culture was studied in terms of increase in fresh weight and dry weights of root callus after every subculture. Leaf callus initiation was best with the hormone combinations, (MS + IAA + 2, 4-D 1 ppm each) in 15 days, and they were not maintained further as the initiated callus along with leaf explants was used for initiation of hairy roots.

Initiation of Hairy Root Culture: The hairy root culture was initiated from root callus culture with hormone combination MS + IAA + BA + 2, 4-D (0.5 ppm + 1 ppm + 0.5 ppm) in 20 days treated with strain MTCC-532 **Fig. 4**. Earlier reports have shown genetic transformation of Taraxacum platycarpum using *A. rhizogenes* and A. tumefaciens [8, 9] Later on *A. rhizogenes* mediated hairy root induction was reported in *Taraxacum officinale* using strains A4 and ATCC 15834 and sesquiterpene lactones were analyzed by means of HPLC [10]. The strain MTCC-2084 failed to show initiation and growth of hairy roots even after repeated experiments and trying different compositions of media.

FIG. 4: INITIATION OF HAIRY ROOT CULTURE FROM ROOT CALLUS USING HORMONE COMBINATION R1-MS + IAA + BA + 2, 4-D (0.5 PPM + 1 PPM + 0.5 PPM) IN HALF STRENGTH SOLID MS MEDIUM IN 20 DAYS USING STRAIN MTCC-532

Also, hairy root initiation and growth were profuse in half-strength MS medium as compared to the full strength MS medium, and the hairy roots were maintained up to 60 days **Fig. 5-7**. The fresh weights of the hairy roots maintained at various time intervals of 30, 45 and 60 days were recorded as shown in **Table 1**. This difference may be attributed to the difference in concentration of individual components of the growth medium and their role in limiting the biomass of the hairy root cultures [11]. In control explants hairy root formation was not observed. When leaf explant of *Taraxacum officinale* was infected with *Agrobacterium rhizogenes* strains the hairy roots did not initiate even by using the above hormone combination but when the leaf explants with callus initiation were infected with *Agrobacterium rhizogenes* hairy root initiation was seen hence, leaf explants with initiated callus was a better explant than leaf explants. But the hairy roots initiated from leaf explants with initiated callus failed to grow further in either half strength or full-strength MS medium and other conditions. Therefore, only the explants-root callus culture maintained on hormone combination- MS + IAA + BA + 2, 4-D (0.5 ppm + 1 ppm + 0.5 ppm) successfully showed genetic transformation but not the leaf or leaf callus. The growth capacity of hairy roots and their morphological characteristics may be attributed to level of expression of role genes in the genetically transformed roots because variations in role genes expression are responsible for changes in expression of morphological features. The selection of appropriate strain of *Agrobacterium rhizogenes* also plays an important role in success of genetic transformation. The differences observed between the capacity of strains MTCC-2084 and MTCC-532 to induce hairy roots may be attributed to the degree of virulence of these strains and nature of plasmids present in different strains [12]. Also the genetic transformation of the plant species depends on the susceptibility of the plant species towards the acceptance of Ri plasmid in the genetic material of the plant [13]. Also the presence of opines was confirmed in hairy roots by paper electrophoresis when they were compared with standard agropines and mannopines which showed R_f values 0.54 and 0.27 respectively.

FIG. 5: 30 DAYS OLD INDEPENDENT HAIRY ROOT CULTURE IN HALF STRENGTH LIQUID MS MEDIUM INDEPENDENT OF GROWTH HORMONES USING STRAIN MTCC-532

FIG. 6: 45 DAYS OLD INDEPENDENT HAIRY ROOT CULTURE IN HALF STRENGTH LIQUID MS MEDIUM INDEPENDENT OF GROWTH HORMONES USING STRAIN MTCC-532

FIG. 7: 60 DAYS OLD MAINTAINED HAIRY ROOT CULTURE INDEPENDENT OF GROWTH HORMONES IN LIQUID HALF STRENGTH MS MEDIUM

TABLE 1: EFFECT OF MS MEDIUM STRENGTH ON MAINTENANCE OF HAIRY ROOT CULTURES OF TARAXACUM OFFICINALE WEBER

S. no.	Medium	Average weight of hairy root (HR-RC) culture (g)		
		30 days	45 days	60 days
HR-RC	MS ½	3.81 ± 0.02	5.95 ± 0.02	6.93 ± 0.03
	MS	2.66 ± 0.03	-	-

Qualitative Estimation: The qualitative chemical tests were performed for the presence/absence of various plant constituents in the natural root, root callus and hairy root culture of different hormonal combinations. It was found that the natural root, root callus culture and hairy roots contain carbohydrates, tannins, saponins, mucilage, phenolic compounds, alkaloids, amino acids, terpenoids, and proteins.

Quantitative Estimation: Pentacyclic-triterpenes, taraxerol & taraxasterol are therapeutically active biomarker compounds that are widely present in various plants. Rhizophora mangle leaves were reported to show extraordinarily high taraxerol content in its leaves, especially *Rhizophora racemosa* whereas taraxasterol, not exclusively but quite frequently present in compositae family. Taraxasterol was initially explored by Furuno *et al.*, 1993, suggesting the organ mediated accumulation of taraxasterol in *T. officinale*. Again its organ mediated accumulation, especially in latex, was suggested by Akashi *et al.*, 1994. (Pseudo)-laticifer cells were reported to be the probable site of biosynthesis of the taraxastane series of compounds [14].

Both these potential triterpenoids are present in a very lesser quantity. We are here introducing a plant model system to trigger the concentration of both these components for the first time from the hairy root culture of *T. officinale*. RP-HPLC method is one of the most reliable and fast method for identification of phytochemicals. Quantitative analysis of taraxasterol and taraxerol was carried out by RP-HPLC method. The method was validated in accordance with ICH guidelines by determining several parameters of performance quality. The chromatogram showed the retention time of 3.5 min (A: 2140500) with standard taraxasterol and 4.975 (a: 2181000) with standard taraxerol in methanol **Fig. 8** and **9**. The chromatogram of standard and the different extracts showed sharp and uniform peak with nonsignificant variations in retention time.

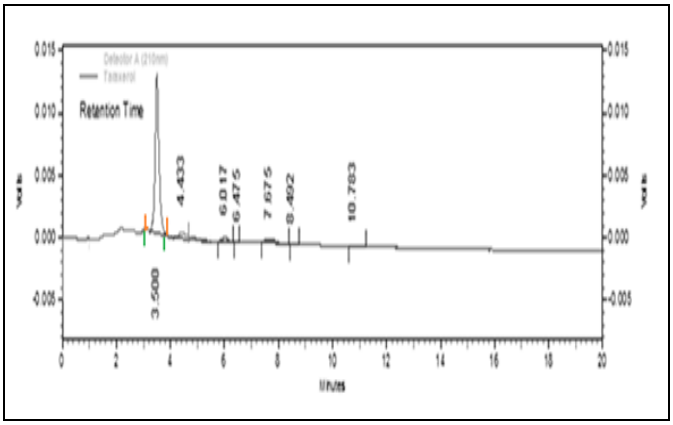

FIG. 8: HPLC PEAK OF TX STANDARD 100 µg/ml

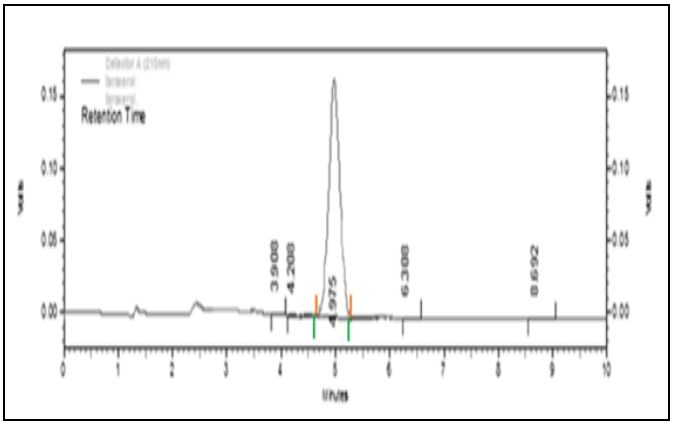

FIG. 9: HPLC PEAKS OF TA STANDARD 100 µg/ml

By HPLC analysis, the quantity of taraxasterol in natural root extract was found to be 2.96 µg/ml and 1.686 µg/ml for taraxerol **Fig. 10**. In contrast root callus cultures, R1 (MS + IAA + BA + 2, 4-D) (0.5ppm + 1 ppm + 0.5 ppm) showed 3.013 µg/ml of taraxasterol 1.757 µg/ml of taraxerol **Fig. 11**. But hairy root culture from root callus showed 8.72 µg/ml of taraxasterol and 5.69 µg/ml of taraxerol **Fig. 12** and **Table 2**. Previous reports suggest the presence of taraxasterol in cultures of *Taraxacum officinale* and root [15], but both these compounds are quantified for the first time from hairy root culture. As it has been reported in the literature that genetic transformation using *Agrobacterium rhizogenes* leads to increased growth and content of phyto-constituents hence, it was justified to undertake genetic transformation studies. In this study, two strains of *Agrobacterium rhizogenes* MTCC-532 and MTCC-2084 were used for initiating hairy roots in T.O leaf and 45 days old root callus initiated and maintained using hormone combination MS + IAA + BA + 2, 4-D (0.5 ppm +

1 ppm + 0.5 ppm) and quantification of taraxasterol (TX) and taraxerol (TA) were done by HPLC method. Only root callus maintained on MS + IAA + BA + 2, 4-D (0.5 ppm + 1 ppm + 0.5 ppm) showed initiation of hairy root culture after infection with *Agrobacterium rhizogenes* strain - 532 having optical density 0.82.

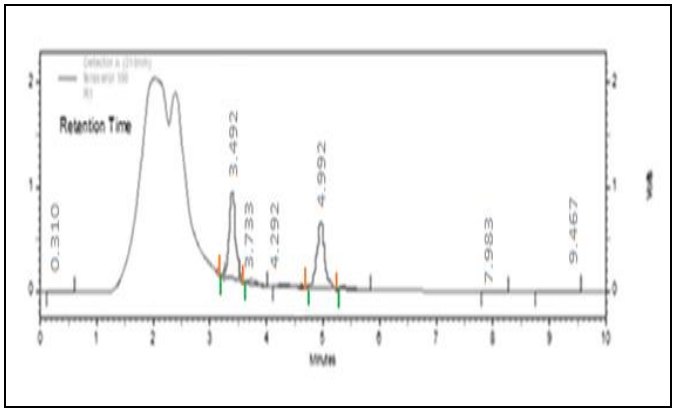

FIG. 10: HPLC GRAPH OF NATURAL PLANT ROOTS (PR) EXTRACT OF T.O

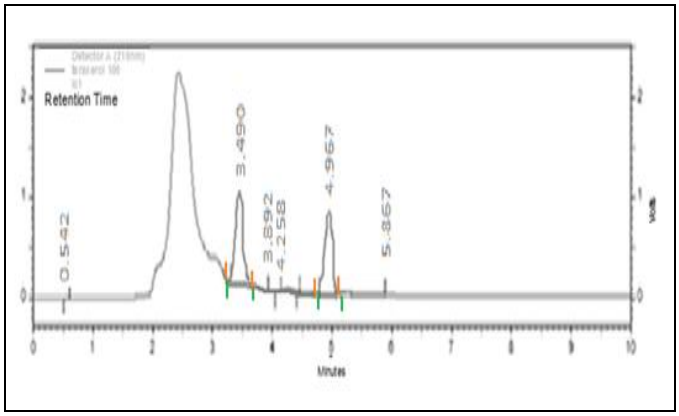

FIG. 11: HPLC GRAPH OF ROOT CALLUS R1- MS + IAA + BA + 2, 4-D (0.5 PPM + 1 PPM + 0.5 PPM)

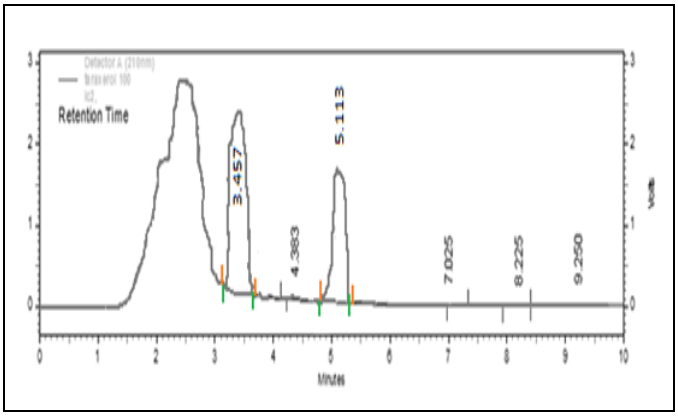

FIG. 12: HPLC CHROMATOGRAM OF HAIRY ROOT CULTURE

The strain of *Agrobacterium rhizogenes* - 2084 did not show hairy root initiation. The initiation of hairy roots was not seen in T.O. leaves even with either of the strains. Paper electrophoresis confirmed the presence of agropines and man opines in the hairy root cultures. Further, the HPLC analysis of hairy roots showed increase of TX content to 0.088% w/w and TA to 0.037% w/w as compared with natural root PR which showed TX to 0.0299% w/w and TA to .0169% w/w **Fig. 13** & **Table 2**. Therefore, the results showed that hairy roots showed four times increase in content of TX and two times increase in TA content.

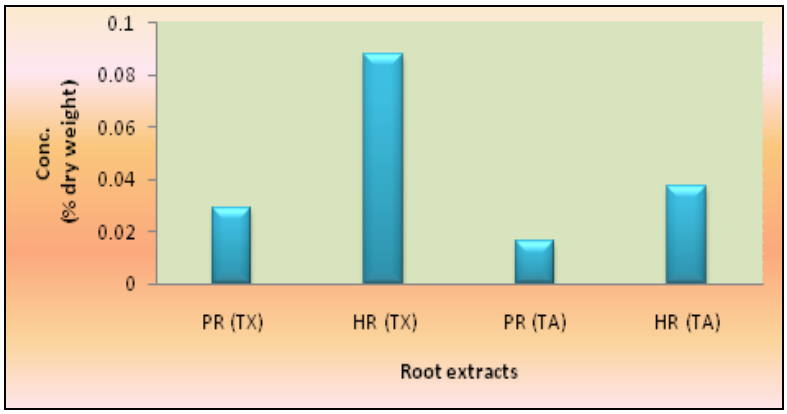

FIG. 13: GRAPHICAL REPRESENTATION OF AMOUNT OF TARAXASTEROL AND TARAXAEROL PRESENT IN HAIRY ROOT CULTURES AS COMPARED WITH PLANT ROOT EXTRACT OF T.O.

TABLE 2: CONCENTRATION OF TX AND TA QUANTIFIED BY HPLC IN HAIRY ROOT CULTURES

S. no.	Tissue / Cell line	Const.	Retention time (min)	Area	Conc. in injected sample (µg/ml)	Conc. (% dry weight)
1	PR	TX	3.492	93321	2.96	0.029
		TA	4.992	71727	1.686	0.016
2	HR	TX	3.457	208921	8.72	0.088
		TA	5.113	151234	3.67	0.037

PR - Plant root, HR- Hairy root from, TX - Taraxasterol, TA - Taraxerol

CONCLUSION: The HPLC analysis showed a marked enhancement in the yield of both these compounds in hairy root culture from root callus as compared to the natural root and root callus. The present work is significant as the two biologically active triterpenols have been quantified simultaneously for the first time in genetically transformed hairy root cultures.

Hence, hairy root culture will prove to be a valuable strategy to increase the yield of these secondary metabolites on a commercial as well as industrial scale. Further, by using advanced plant tissue culture techniques to the hairy root culture, such as precursors and elicitors (biotic and abiotic) addition, will definitely boost the yield of these contents to a considerable level.

ACKNOWLEDGEMENT: One of the authors Kiran Sharma is thankful to CSIR, New Delhi, for granting Senior Research Fellowship.

CONFLICT OF INTEREST: There is as such no conflict of interest among the author.

REFERENCES:

1. Sharma K and Zafar R: Occurrence of taraxerol and taraxasterol in medicinal plants. Pharmacognosy Reviews 2015; 9: 19-23.
2. Martinez M, Poirrier P, Chamy R, Prufer D, Schulze-Gronover C and Jorquera LRG: *Taraxacum officinale* and related species - an ethnopharmacological review and its potential as a commercial medicinal plant. Journal of ethnopharmacology 2015; 169: 244-62.
3. Vladimirov Y, Mateeva TV and Lutova LA: Opine biosynthesis and catabolism genes of *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*. Russian Journal of Genetics 2015; 51(2): 137-46.
4. Korde VV, Dhas SS and Gurave NN: Hairy root culture: a promising approach in biotransformation. Asian Journal of Plant Science and Research 2016; 6: 6-11.
5. Aleksey K, Bulat K, Zilya V, Gulnar Y and Aleksey C: *A. rhizogenes* mediated hairy root induction in *P.andersonii* Planch. Asian Journal of Plant Sciences 2017; 16: 227-34.
6. Sharma K and Zafar R: Simultaneous estimation of taraxerol and taraxasterol in root callus cultures of *Taraxacum officinale* weber. Int Journal of Pharma and Phytochemical Research 2014; 6: 540-46.
7. Shivbalan K, Gadamchetty P, Selvam S, Ramamoorthy S, Periyasamy SK and Markandan M: Genotype independent and enhanced in planta *Agrobacterium tumefaciens*-mediated genetic transformation of Peanut. 3 Biotech 2018; 8(4): 202.
8. Lee MH, Yoon ES, Jeong JH and Choi YE: *Agrobacterium rhizogenes* mediated transformation of *Taraxacum platycarpum* and changes of morphological characters. Plant Cell Reports 2004; 22: 822-27.
9. Bae TW, Park HR, Kwak YS, Lee HY and Ryu SB: *A. tumefaciens* mediated tansformation of medicinal plant *T. platycarpum*. Pla Cell Tiss and Organ Cul 2005; 80: 51-57.
10. Mahesh A and Jeyachandran R: *Agrobacterium-rhizogenes* mediated hairy root induction in *Taraxacum officinale* and analysis of sesquiterpene lactones. Plant Biosystems 2011; 145: 620-26.
11. Miroshnichenko D, Ashin D, Pushin A and Dolgov S: Genetic transformation of einkorn (*T. monococcum* L), a diploid cultivated wheat species. BMC Biotechnology 2018; 18: 1-13.
12. Trovato M, Mattioli R and Costantino P: From *A. rhizogenes* RolD to plant P5CS: exploiting proline to control plant development. Plants 2018; 108: 1-13.
13. Gai QY, Jia J, Leu M, Wei ZF, Zu YG, Ma W and Fu YJ: Establishment of hairy root cultures by *Agrobacterium rhizogenes* mediated transformation of *Isatis tinctoria* L. for efficient production of flavonoids and evaluation of anti-oxidant activities. PLOS 2018; 10: 1-15.
14. Basyuni M, Tsujimoto E, Baba S, Oku H, Kinjo K and Takara K: Triterpene synthases from the okinawan mangrove tribe. Rhizophoraceae 2007; 274: 5028-42.
15. Sharma K and Zafar R: Optimization of methyl jasmonate and β-cyclodextrin for production of taraxerol and taraxasterol in *Taraxacum officinale* weber cultures. Plant Physiology and Biochemistry 2016; 103: 24-30.

How to cite this article:
Sharma K: Analysis of taraxerol and taraxasterol in hairy root cultures of *Taraxacum officinale* weber. Int J Pharm Sci & Res 2019; 10(11): 5094-02. doi: 10.13040/IJPSR.0975-8232.10(11).5094-02.