

Full Length Research Paper

Co-culturing of *Catharanthus roseus*, *Vinca major* and *Rauwolfia serpentina* cell suspensions in shake flask and bioreactor: Production of a novel alkaloid with antioxidant potential

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Conditions for co-culturing the cell suspensions of *Catharanthus roseus*, *Rauwolfia serpentina* and *Vinca major* in shake flask and bioreactor are described here for the possible complementation of the terpenoid indole alkaloid pathway operating in them. *Catharanthus* + *Rauwolfia* or *Catharanthus* + *Vinca* cell combinations could be reared on a Murashige and Skoog medium containing 2.0 mg L⁻¹ naphthalene acetic acid (NAA) and 0.2 mg L⁻¹ kinetin (Kn). A 20- and 40-fold increment in the biomass of these co-cultures was achieved within 30 days in a stirred tank bioreactor. Thin layer chromatography (TLC) of the alkaloid extracts of co-cultures of *Rauwolfia* + *Catharanthus* showed the presence of two novel compounds (RF1 and RF2) upon staining with Dragendorff's reagent. Proton nuclear magnetic resonance (¹HNMR), electrospray ionization mass spectrometry (ESI-MS) and ultraviolet (UV) spectral analysis data of these compounds suggested an indole alkaloid identity. Out of these two alkaloids, compound RF1 was found to possess strong antioxidant potential.

Key words: *Catharanthus roseus*, *Rauwolfia serpentina*, *Vinca major*, co-culture, terpenoid indole alkaloids, pathway complementation.

INTRODUCTION

Plant cell, tissue and organ cultures have been widely explored as viable renewable resource for developing effective *in vitro* production platforms for a variety of high-value plant secondary metabolites of commercial importance (Alfermann and Peterson, 1995; Collin, 2001; Zhong, 2001; Rao and Ravishankar, 2002; Verpoorte et al., 2002; Mulabagal and Tsay, 2004; Weathers et al.,

2010). However, barring a few successful cases of industrial scale utilization for shikonin, berberine, ginsenosides and rosmarinic acid production (Ulbrich et al., 1985; Fujita, 1988; Smith, 2005; Dornenburg, 2008), the biomass and/or metabolite productivity levels in majority of such endeavors has been generally low and inconsistent. Genetic instability, incomplete expression of the targeted metabolic pathway due to silencing of certain differentiation-linked key regulatory enzymes/intermediates under *in vitro* conditions, insufficient sink capacity and difficulties associated with process up-scaling are the major limitations of these production systems. Meanwhile, two major experimental strategies are being

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followed to address these bottlenecks: (i) selection of high-yielding cell lines and optimization of their growth and production kinetics in terms of nutritional, hormonal, elicitation and physical requirements and (ii) tailoring of targeted metabolic route by genetic engineering tools to either hyper-express a limiting pathway enzyme(s) or to silence the diversion of a common precursor/intermediate towards cross-linked sub-way to achieve improved biochemical flux towards the intended metabolic route (Verpoorte and Alfermann, 2000; Zhong, 2001; Verpoorte et al., 2002; Weathers et al., 2010; Verma et al., 2012).

Another line of investigation that has been recently proposed in this direction is the inter- and intra-species co-culture systems for obtaining higher biomass and/or metabolite production via pathway complementation (Sidwa-Gorycka et al., 2003; Luczkiewicz and Kokotkiewicz, 2005; Wu et al., 2008; Yue et al., 2010). This approach basically works on the presumption that metabolites produced by tissue culture of one plant species upon their release into the medium is utilized by the different tissues of same or other plant species for further conversion into a more useful end product. The technique is more likely to be successful in combination of cells and tissues that share a common biosynthetic pathway till terminal diversions step to produce species- or tissue-specific products. Subroto et al. (1996) demonstrated for the first time that co-cultures of hairy roots and shooty teratomas of *Atropa belladonna* led to higher scopolamine recovery from the shoot tissue in comparison to their mono-cultures. The hairy roots in such co-cultures released more hyoscyamine in the medium, which in turn was taken up by the shoots for its final conversion into scopolamine due to higher activity of the enzyme 6- β -hydroxyhyoscyamine in them. Sidwa-Gorycka et al. (2003) studied the co-culture of *Ammi majus* hairy roots with cell suspension or shoot cultures of *Ruta graveolens* and recorded better shoot biomass and 2.5-folds more xanthotoxin production in root-shoot combination. Luczkiewicz and Kokotkiewicz (2005) also used hairy root and shoot co-cultures of *Genista tinctoria* for higher accumulation of phytoestrogenic isoflavones genistin, diadzin and diadzein in the shoot cultures. Increase synthesis of these isoflavones was accounted for by the release of their precursor molecule, isoliquiritigenin, from the hairy roots when ascorbic acid was added into the medium after 42 days of culture growth in a basket bubble bioreactor. Isoliquiritigenin which is otherwise not present in shoots is utilized by the co-cultured shoots to synthesize 38 times more daidzin.

Furthermore, Wu et al. (2008) grew adventitious roots of *Panax ginseng* and *Echinacea purpurea* in co-cultures in different proportions and found that 4:1 and 3:2 inoculum ratios of *Panax* and *Echinacea* roots favored higher ginsenoside accumulation in the mixed cultures, whereas higher proportion of *Echinacea* roots (2:3 inoculum density) resulted in better recovery of caffeic acid derivatives. Pathway complementation study by co-

culturing of cell suspension cultures of two different plant systems is so far confined to just one report of Yue et al. (2010), wherein two-folds improvement in the production of Rb1 ginsenoside (51.5 mg/L) was recorded when *Amaranthus tricolor* cells were added in 16 days old cell suspensions of *P. ginseng* followed by incubation for four more days in the mixed culture mode. Improved Rb1 ginsenoside production was traced to 1.6 times higher activity of the enzyme Rb1 synthase in *Panax* cells due to the uptake of pathway intermediate squalene released into the medium by *A. tricolor* cells. An atypical co-culture system of *Taxus chinensis* cell suspension with its endophytic fungus *Fusarium marirei* leading to improved paclitaxel production has also been recently reported (Li et al., 2009).

The present study was undertaken to advance this subject by exploring the possibility of up-regulating terpenoid indole alkaloids (TIAs) biosynthetic pathway in the co-cultured cell suspensions of *Catharanthus roseus* with that of *Rauwolfia serpentina* or *Vinca major* plants. These three plant species of the family Apocynaceae share the common TIAs pathway up to the formation of the central precursor intermediate Strictosidine (Figure 1) before diverting into different routes to form a wide range of corynantheine, iboga and aspidosperma types of alkaloids (Verma et al., 2012). Many of the biosynthesized TIAs of these plant species are in use as clinical drugs (Cordell et al., 2001; De Luca and Laflamme, 2001; Van der Heijden et al., 2004). For example, the anti-mitotic alkaloids vincristine and vinblastine obtained from *C. roseus* leaves are indispensable components of chemotherapeutic regimens against a variety of cancers (Arora et al., 2010), whereas ajmaline, serpentine and reserpine alkaloids present in *R. serpentina* roots are important anti-hypertensive and anti-arrhythmic drugs (Itoh et al., 2005). *V. major* leaves on the other hand are primary source of a TIA vincamine, which is widely prescribed as a vasodilatory agent in modern and traditional systems of medicine (Rahman et al., 1995). Cell suspension cultures of these plant species have been frequently tested for their biosynthetic capabilities, but the yield of the desired alkaloids has always found to be very low because of a rigid temporal and spatial regulation of the TIAs pathway in these plants. In this communication, we reported for the first time conditions for the successful establishment of co-cultures of *C. roseus* cell suspension with *R. serpentina* and *V. major* cells, leading to the recovery of two novel TIAs with strong antioxidant activities.

MATERIALS AND METHODS

Raising cell suspensions and microscopic studies

Leaf callus cultures of the *R. serpentina*, *V. major* and *C. roseus* were initiated and maintained on Murashige and Skoog (1962) medium supplemented with 2.0 mg L⁻¹ α -naphthalene acetic acid

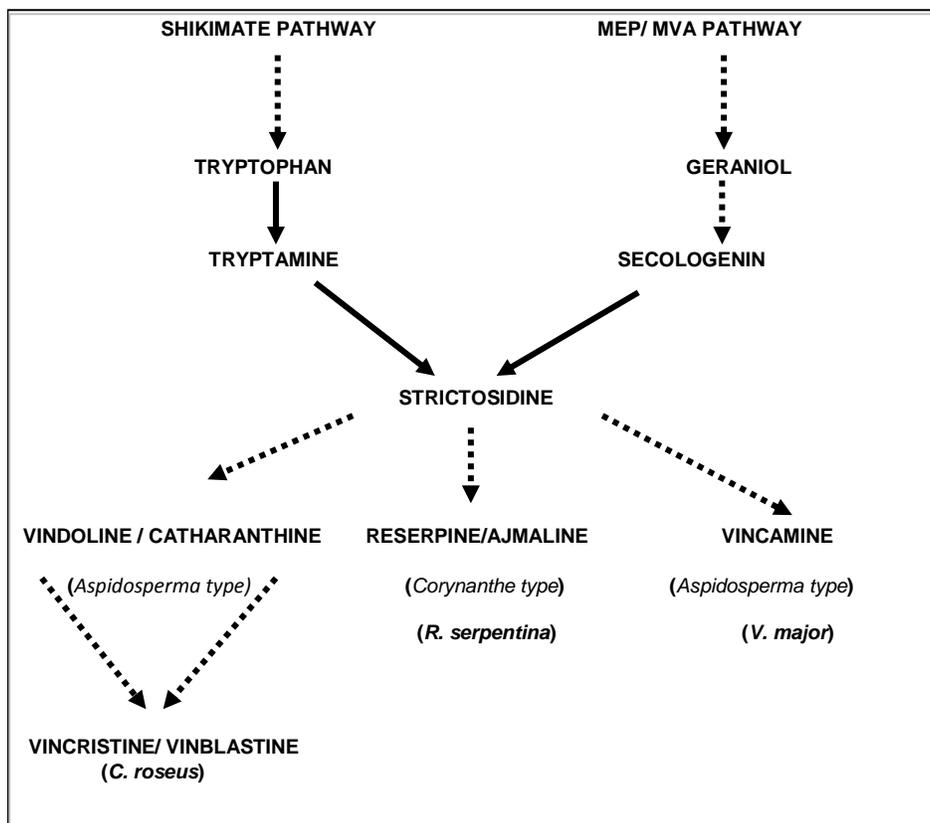


Figure 1. Strictosidine as a universal precursor of different classes of terpenoid indole alkaloids in the test plant systems used.

(NAA) + 0.5 mg L⁻¹ 6-benzylaminopurine (BAP); 1.0 mg L⁻¹ 2, 4-dichlorophenoxyacetic acid (2,4-D)+ 0.5 mg L⁻¹ BAP; MS+ 2.0 mg L⁻¹ NAA + 0.2 mg L⁻¹ kinetin (Kn), respectively. Cell suspensions were raised from the induced callus on the respective liquid medium of similar compositions. For initiating the co-cultures of *C. roseus* + *R. serpentina* and *C. roseus* + *V. major*, 200 mg of cell inoculum from 30 days old suspensions of each plant system were mixed in 1:1 ratio and incubated in 40 ml of MS + 2.0 mg L⁻¹ NAA + 0.2 mg L⁻¹ Kn containing liquid medium.

To monitor the relative population of different partner cells during co-culturing, the cells were differentially stained with rhodamine isothiocyanate (RITC) or fluorescein isothiocyanate (FITC) dyes before mixing. Stock of FITC/ RITC was prepared by dissolving 10 mg salt into 1 ml ethanol. In brief, 100 µL of this stock was used to stain the cell inoculums in 40 ml medium. Cell densities and morphological features of partner cells were recorded and photographed using a UV microscope (Leica DMLB) fitted with COHU high performance CCD camera.

Growth kinetic studies and bioreactor up-scaling

The growth kinetics of individual and mixed cell cultures was studied in shake flasks at 10 days intervals through a 40 days incubation cycle. Biomass accumulation was tabulated as percent increase over the initial inoculum. Data was represented as mean performance of three replicated cultures per duplicated experiment. The resultant co-cultures of *Catharanthus* + *Rauwolfia* and *Catharanthus* + *Vinca* were also scaled up in a 7 L stirred

tank bioreactor (Model ADI-1010; Applikon Biotechnology Holland). The parameters employed during bioreactor up-scaling included air flow rate of 6 L min⁻¹ equivalent to 60% dissolved oxygen (DO₂). The cultures were aerated through a sintered steel sparger and cell mixing was facilitated by a Rushton-type impeller.

Chemical analysis

The total terpenoid indole alkaloids (TIAs) concentration in the cultured cells was determined by extracting 1 g oven-dried (40 - 50°C) tissues with methanol (3 × 30 ml; 12 h each) at room temperature. The methanol (MeOH) extracts were pooled and reduced to 10 ml in vacuum, mixed with 10 ml dH₂O, acidified with 10 ml of 3% hydrochloric acid (HCl) and washed thrice with n-hexane (3 × 30 ml). The aqueous portion was further extracted with chloroform (3 × 30 ml), washed with dH₂O, dried over anhydrous sodium sulphate, concentrated in vacuum and weighed to determine total alkaloids content. Thin layer chromatography (TLC) of the crude alkaloid extracts was carried out using silica plates (MERCK aluminium/ glass sheets; 20 × 20cm; silica gel 60F₂₅₄) as per the procedure of Hernandez-Dominguez and Flota (2006). The mobile phase used consisted of 12% methanol in chloroform with 2 - 3 drops of ammonia. Spots were resolved by spraying the plates with Dragendroff's reagent and compared with those of standard reference compounds of ajmalicine, serpentine, catharanthine, vindoline, vincamine, vincristine and vinblastine (Sigma-Aldrich, USA and Tauto Biotech, China).

The two prominent spots of novel TIAs obtained in the extracts of

co-cultured cells that did not correspond to the TLC profiles of either of the partner cells were isolated by means of preparative TLC. For chemical characterization, these compounds were subjected to proton nuclear magnetic resonance spectroscopy ($^1\text{H NMR}$; C^{13}), ultraviolet (UV) absorption spectra and electrospray ionization mass spectrometry (ESI-MS). For NMR analysis, 20 mg of compound was dissolved and evaporated 2 - 3 times in carbon tetrachloride (CCl_4) to remove the solvent protons and finally dissolved in deuterated chloroform (CDCl_3). Similarly, 1 - 2 mg of the isolated compound was used in the mass and UV analysis. NMR analyses were done on a Bruker Avance 300 MHz instrument with TMS as an internal standard. ESI-MS spectra were recorded on Applied Biosystems API-300.

***In vitro* antioxidant assay**

The isolated compounds were dissolved in dimethylsulphoxide (DMSO) to a final concentration of 0.01%. Ferric reducing antioxidant power (FRAP), 1,1 diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, total phenolic content (TPC) in terms of gallic acid equivalence, total antioxidant capacity (TAC) and reducing power (RP) assays were performed by standard reported protocols (Luqman et al., 2009; Gulcin et al., 2010; Serbetci and Gulcin, 2010). The results are given as mean \pm standard deviation (SD) of three independent experiments in replicate. Correlation among the antioxidant parameters was done by determining Pearson's coefficient using software SYSTAT11.

RESULTS AND DISCUSSION

Establishment of cell suspension cultures

Growth kinetics of cell suspension cultures of *C. roseus*, *R. serpentina* and *V. major* showed a typical sigmoid pattern in their respective medium (Figure 2a). In comparison to *C. roseus* cells, both *R. serpentina* and *V. major* cells recorded a longer lag phase of around 20 days with a growth index of only 76.23 and 44.24, respectively on 20th day of the culture cycle. The growth index of *C. roseus* cultures reached 178.4 by this time. While subsequently the *R. serpentina* cells showed rapid exponential biomass accumulation to acquire a growth index of 540.67 on 30th day, *V. major* cells continued to grow slowly and attained a GI of only 193.5 on the 40th day of incubation. Both *R. serpentina* and *C. roseus* cultures maintained a steady growth stage between 30th and 40th day of the culture cycle before entering the declining phase. Since subsequent co-culturing was intended to be carried out in MS + 2.0 mg L⁻¹ NAA + 0.2 mg L⁻¹ Kn in which *C. roseus* cells grow optimally, the growth kinetics of *R. serpentina* and *V. major* cells was also checked in this medium (Figure 2b). It was observed that *R. serpentina* cells though took a longer time to acclimatize in the new medium with a GI of only 308.12 on 30th day, showing rapid biomass gain during next 10 days to acquire even a marginally improved growth index of 693.7 in comparison to 610.5 on the 40th day in their original NAA/BAP containing growth medium. *V. major* suspension on the other hand, exhibited a rapid adjustment in NAA/ Kn medium of *C. roseus* and

continued to grow exponentially in this medium with growth indices of 394.01 and 592.55 after 30th and 40th day of culture in comparison to corresponding growth indices of 95.28 and 193.45 in the original 2, 4-D/ BAP medium. Microscopic examination of cell suspensions of the three intended partners when grown singly in NAA/Kn containing *C. roseus* medium revealed that while *C. roseus* cells had an elongated and globular morphology with occasional presence of differentiated thick walled trachiedal structures (Figure 3a and b), the cells of *V. major* were oval with a thin smooth cell wall, whereas *R. serpentina* cells exhibited a more clustered and granular phenotype (Figure 3c and d). RITC and FITC staining of these cultured cells (Figure 3e and f) helped in tracking their ontogeny in cultures.

Co-culturing of *C. roseus* + *V. major* and *C. roseus* + *R. serpentina* cells was initiated using 1:1 inoculum ratio in 40 ml medium. *C. roseus* + *V. major* co-cultures grew better than that of *C. roseus* + *R. serpentina* combination. While the former could register a growth index of 542.28 on the 40th day, the later combination showed lot of cells death of both the partners during initial 20 days of incubation period, resulting in a final growth index of only 292.29 on the 40th day. Microscopic examination of the 30th and 40th day old cultures in case of *C. roseus* + *V. major* (Figure 3g) combination indicated that 1:1 frequency of the two partner cells was steadily maintained throughout the culturing period, whereas in case of *Catharanthus* + *Rauwolfia* combination this ratio was 3:2 (Figure 3h). When co-cultures of both the combinations were sub-cultured onto the fresh medium, they showed gradual increase in their growth rates and after 4 - 5 cells doubling passages they acquired a consistent growth index of around 550. The mutual influence of partner cells and/or tissues on growth in co-cultures was also observed in many earlier studies (Sidwa-Gorycka et al., 2003; Wu et al., 2008; Yue et al., 2010) and this was often traced to the time required by partner systems for cellular adjustment in a new neighborhood under the culture environment. Such mutual effect of the partner cells were also found to be influenced by their mixing ratios at the beginning of the culture cycle. This was very clearly displayed by root co-cultures of *P. ginseng* and *E. purpurea* where it was found that while initial inoculum ratios of 4:1 and 3:2 of these tissue partners favored highest biomass accumulation and ginsenoside accumulation, high inoculum weight of *Echinacea* roots did not allow *Panax* roots to grow and only caffeic acid derivatives were accumulated in the resultant co-cultures (Wu et al., 2008).

The co-cultures of *Catharanthus* + *Rauwolfia* and *Catharanthus* + *Vinca* obtained in the present study were also scaled up in a 7 L stirred tank bioreactor (Figure 3i to l). A 20- to 25-fold increment in biomass (Final harvest weight= 600-630 g; initial inoculum weight (wt) = 40 - 50 g 3 L⁻¹ medium) with a cell pack volume (CPV) = 47 ml 100 ml⁻¹ was achieved through a 30 days cycle in case of

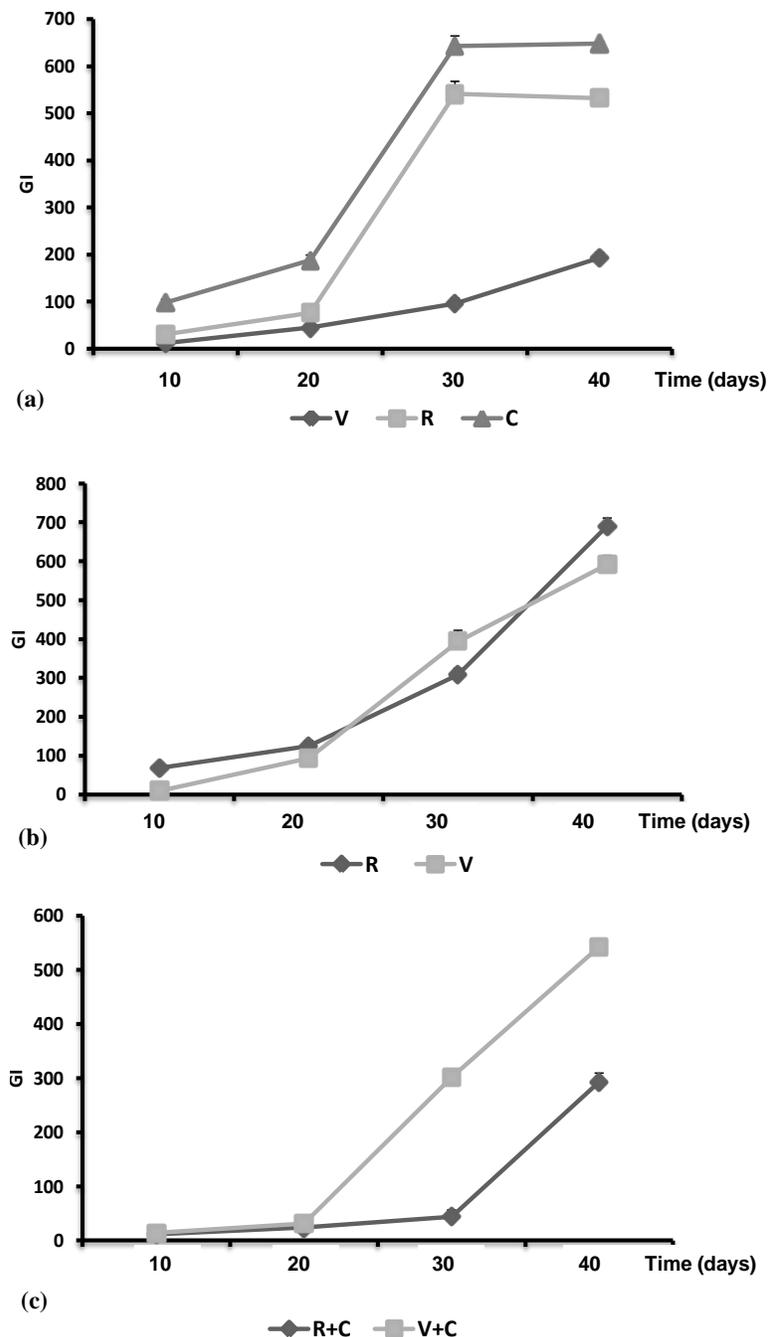


Figure 2. Time-course growth indices (GI) of cell suspensions of three plant species when grown singly or in combinations. (a) *C. roseus* (C), *R. serpentina* (R) and *V. major* (V) alone on their respective control medium; (b) *R. serpentina* and *V. major* cell suspension alone on *Catharanthus* suspension medium; (c) Co-cultures of *C. roseus* cells with *R. serpentina* (C+R) and *V. major* (C+V).

Catharanthus + Rauwolfia co-culture. In case of *Catharanthus + Vinca* combination, an increment of 40-fold was noticed (Final harvest = 1130 g; CPV = 72 ml 100 ml⁻¹). Up-scaling of co-cultures in bioreactor has so far been demonstrated in only one previous study

(Luczkiewicz and Kokotkiewicz, 2005). These workers used a bubble shaped glass air-lift bioreactor with a steel basket inside the glass vessel to keep the shoots and hairy roots of *G. tinctoria* physically separated during the co-cultivation phase. Bioreactor up-scaling using cell

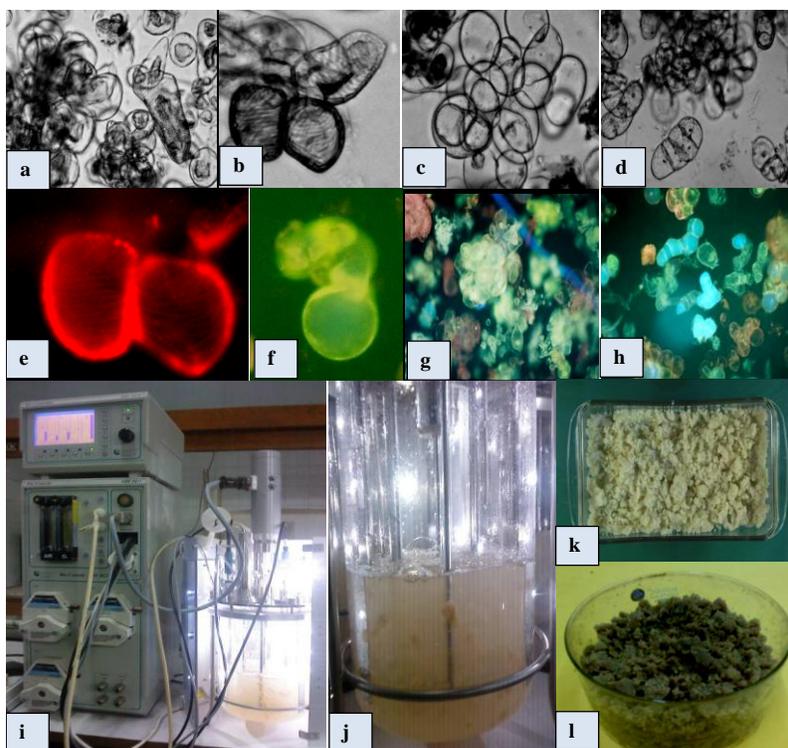


Figure 3. Cells of *C. roseus* (a and b), *R. serpentina* (c) and *V. major* (d) growing individually in suspension cultures, note the presence of thick walled cells (b) in *C. roseus* cultures; cells with RITC (e) and FITC (f) staining when viewed under UV light; Co-cultures of *Vinca* + *Catharanthus* (g) and *Rauwolfia* + *Catharanthus* (h); bioreactor up-scaling of *Rauwolfia* + *Catharanthus* combinations (i and j); cell biomass of *Vinca* + *Catharanthus* (k) and *Rauwolfia* + *Catharanthus* (l) harvested after 30 day growth cycle in bioreactor.

suspensions, as was employed in our study, was found more convenient and easy with no extra effort to create physical barrier to avoid intermingling of organized organs like roots and shoots.

Chemical analysis

The total alkaloid contents of individual and co-cultured cells of *Vinca*, *Catharanthus*, *Rauwolfia*, *Vinca* + *Catharanthus* and *Rauwolfia* + *Catharanthus* were found to be 0.26, 0.31, 0.34, 0.28 and 0.42% dry wt., respectively. TLC analysis of these crude alkaloid extracts did not show any prominent spot with Dragendorff's reagent, except in the case of *Rauwolfia* + *Catharanthus* combination. HPLC analysis of this extract though also did not show the presence of any major TIAs, but indicated two prominent peaks of unidentified alkaloids which were absent in the extracts of *Rauwolfia* or *Catharanthus* cells alone. Spots corresponding to these two unknown peaks were cut from preparative TLC and isolated as pure compounds. ^1H NMR analysis of the two isolated compounds suggested they belonged to indole alkaloid group. Judging from their ^1H NMR, ESI-MS

and UV analysis (Figures 4 and 5), the following information regarding their structure was deduced:

Compound1 (RF1): $R_f = 0.8$ [silica gel, solvent 10% (MeOH)] λ_{max} : 203, 219 and at 270 nm corresponding to indole chromophore. It is an indole alkaloid although there is the absence of N-proton, so it means there must be substitution on N-atom and there are four aromatic protons in the range of δ 7.158 - 7.642. The presence of a $-\text{CH}_3$ proton at 0.941 range and its triplet multiplicity indicates that mode of attachment to a methylene group. The pattern is similar to that of catharanthine. The ESI-MS (positive mode) spectra showed major molecular ion peak at $m/z = 351$.

Compound2 (RF2): $R_f = 0.34$ [silica gel, solvent 15% (MeOH)] λ_{max} : 201, 225 and 275 nm corresponding to indole chromophore. The ^1H NMR spectra shows the signals of aromatic methoxyl ($-\text{OCH}_3$) at δ 3.81 ppm, $-\text{N}-\text{CH}_3$ group at δ 3.25 and 3.2, $-\text{O}-\text{CO}-\text{CH}_3$ (ester function) at $\delta = 3.46$, three aromatic proton ranges from δ 7.16 - 7.18 ppm; δ 3.25 (3H, S, $-\text{N}-\text{CH}_3$); δ 3.46 (3H, S, $-\text{COO}-\text{CH}_3$); δ 3.81 (3H, S, OCH_3); δ 2.29 (3H, S, $\text{O}-\text{COO}-\text{CH}_3$). All the four $-\text{CH}_2$ protons comes in the region from δ 1.63

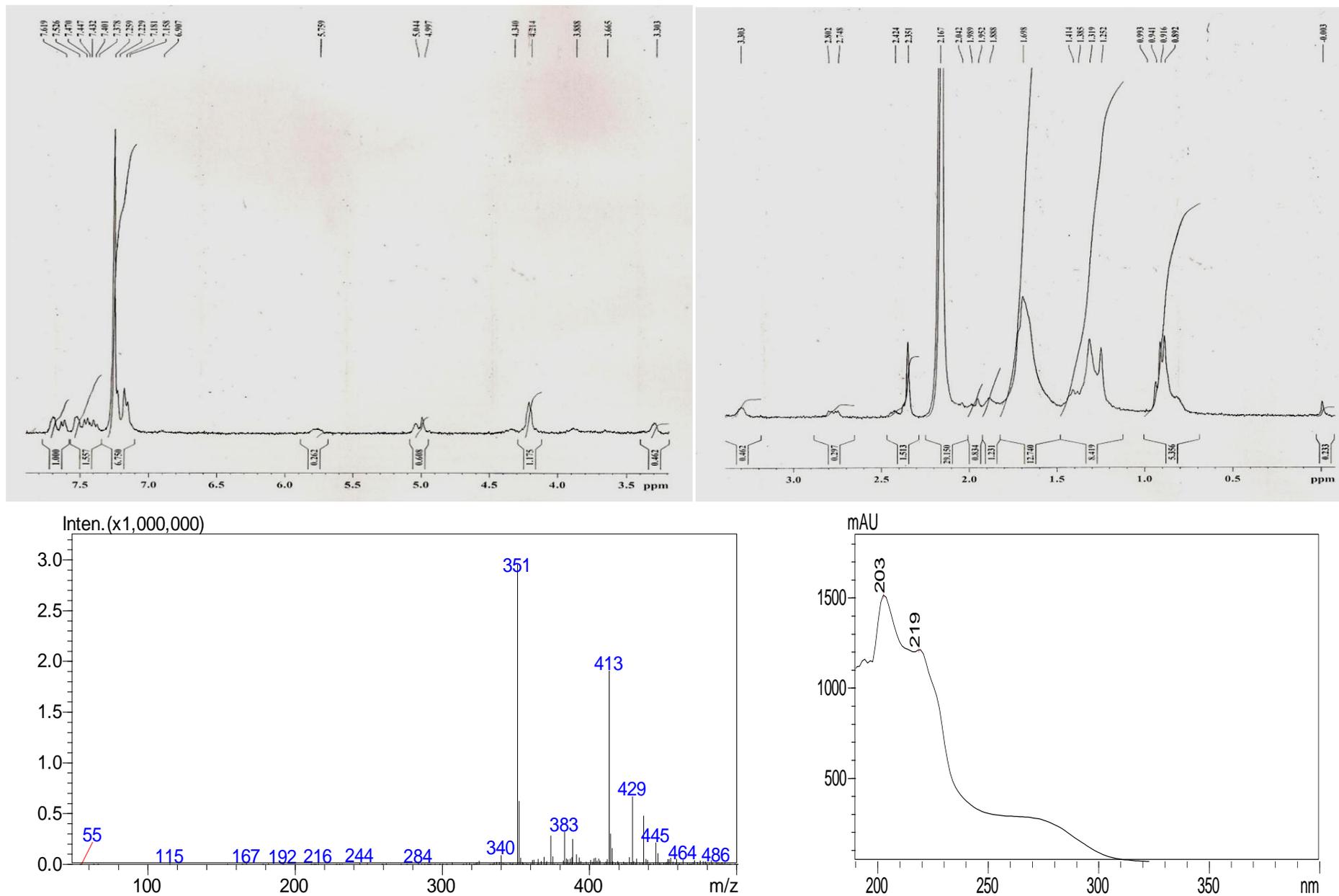


Figure 4. ¹H NMR (a), ESI⁺ Mass (b) and UV (c) spectra of compound RF1 isolated from the alkaloid extract of co-culture of *Catharanthus* + *Rauwolfia*.

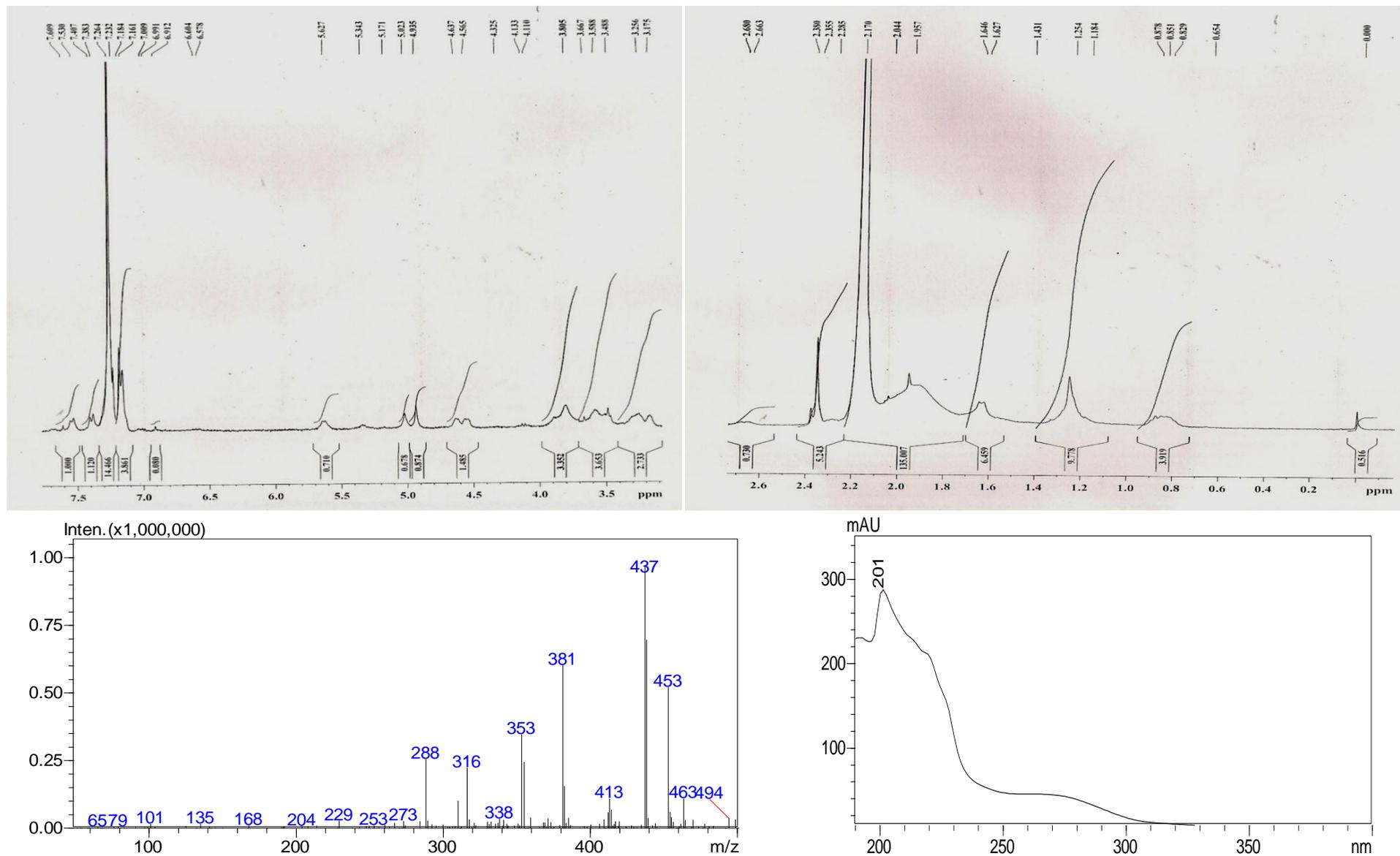


Figure 5. ¹H NMR (a), ESI+ Mass (b) and UV (c) spectra of compound RF2 isolated from the alkaloid extract of co-culture of *Catharanthus* + *Rauwolfia*.

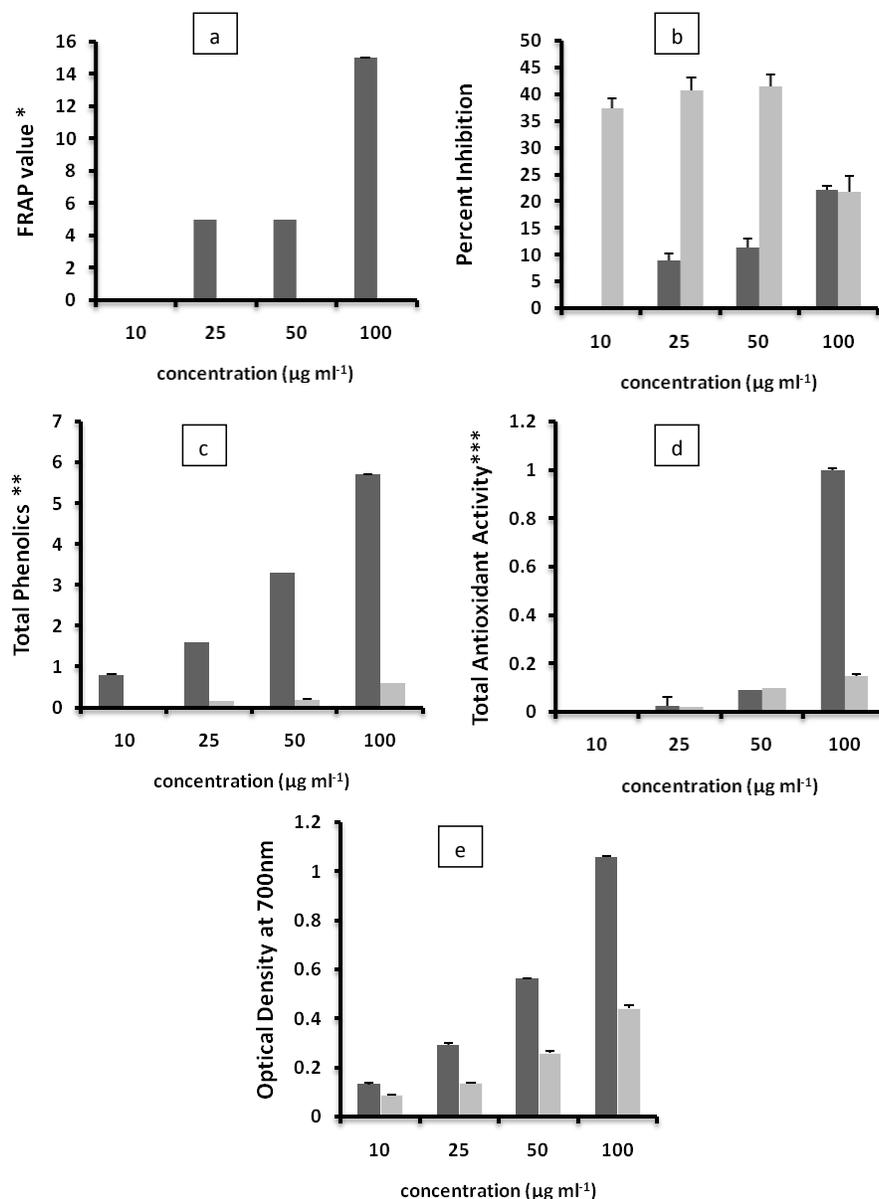


Figure 6. Concentration-dependent antioxidant potential of compounds RF1 and RF2 in FRAP (a); DPPH (b); total phenolics (c); total antioxidant (d) and reducing power (e) estimation *in vitro* assays. *Value expressed as ferrous sulphate equivalence; **estimated in terms of gallic acid equivalence; *** determined in terms of ascorbic acid equivalence; ■ RF1; □ RF2.

to 1.96 and the 1 methyl group appears in the region between δ 0.83 to 0.88. The proton spectrum also showed the presence of hydroxyl group in the molecule which showed signal at δ 3.4 in proton and δ 77.0 in carbon NMR. The ESI-MS (positive mode) spectra showed major molecular ion peak at $m/z = 437$.

Antioxidant activity of the novel alkaloids

Ferric reducing, free radical scavenging and total anti-

oxidant ability of the two isolated novel alkaloids obtained from co-cultures of *Catharanthus* and *Rauwolfia* were tested by performing *in vitro* FRAP, DPPH, TPC, RP and TAAC assays, and the data is summarized in Figure 6. It was observed that the anti-oxidant potential of these compounds increased with concentration. Ferric reducing anti-oxidant power of RF1 (expressed in terms of ferrous sulphate μ M equivalence) also increased with concentration increment (Figure 6a), but RF2 showed no FRAP value any concentration tested. Both the compounds, however, reduced the stable radical DPPH to yellow

colored diphenylpicrylhydrazine. In addition, at 1.0 mg/ml concentration both RF1 and RF2 recorded identical scavenging potential (Figure 6b). A Pearson's coefficient analysis of the data indicated a strong positive correlation (+0.88) between DPPH and FRAP for compound RF1 but not for RF2 (-0.01).

The total phenolic content and total anti-oxidant capacity were also estimated and was found much higher in RF1 at a final concentration of 0.1 mg/L (Figure 6c and d). A strong positive correlation also existed between TPC- FRAP (+ 0.947) and TPC-DPPH (+0.855) for compound RF1, but there was negative correlation in TPC-FRAP (-0.01) and TPC-DPPH (-0.886) for compound RF2. These observations are in agreement with previously published reports (Veliloglu et al., 1998; Holasova et al., 2002; Luqman et al., 2009) and clearly suggested that high phenolic content increases the anti-oxidant activity and there is a linear correlation between these two parameters. The correlation value between TAC, FRAP, DPPH and TPC was 0.943, 0.679 and 0.909, respectively for RF1, whereas there was negative correlation between TAC and DPPH (-0.7) but positive in case of TAC and TPC (+0.875) for RF2. The assessed correlation of RP with FRAP, DPPH, TPC and TAC for RF1 was 0.962, 0.854, 0.998 and 0.929, respectively (Figure 6e). These results clearly indicate that compound RF1 possessed better antioxidant potential than RF2.

Conclusion

Inter- and intra-species cell, tissue or organ co-culture systems constitute a relatively new dimension of studying *in vitro* secondary metabolism to improve the production of plant-based natural products. Efforts so far made in this new direction have mainly revolved around co-culturing of adventitious or transformed hairy roots with shoot cultures of same or different plant species to obtain higher yield of a metabolite that is otherwise produced at very low concentration in one of the partner tissue type. Our study constitutes only the second report wherein co-culturing of cell suspensions of two different plant species was attempted. In an earlier such attempt, the cell suspensions of *P. ginseng* and *A. tricolor* were tested to obtain high accumulation of ginsenoside Rb1 in the *Panax* cells (Yue et al., 2010). Interestingly, the present study has also highlighted for the first time that co-culturing approach can also lead to the recovery of novel metabolites that are otherwise not synthesised by the partner cells when grown independently in culture. The successful demonstration of the possibility of raising co-cultures of *C. roseus* cell suspensions with those of *R. serpentina* and *V. major* has therefore opened a new avenue of research to address the long-pending problem of low expression of terpenoid alkaloid pathway in cell cultures of these plant systems through pathway complementation.

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