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Tissue specific expression of Anthraquinones, flavonoids and phenolics in leaf, fruit and root suspension cultures of Indian Mulberry (*Morinda citrifola* L.)

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Abstract

The cell suspension cultures were established from leaf, fruit and root explants of Indian Mulberry (*Morinda citrifolia*) for the production of medicinally important secondary metabolites, anthraquinones, flavonoids and phenolics and the effect of subculture on these secondary metabolites was studied. The MS medium formulated with NAA, BA and Kinetin was found to be effective to produce anthraquinones as well as flavonoids and phenolics. The presence of these compounds was confirmed by thin layer chromatography and phytochemical screening methods. The results showed that the leaf, fruit and root suspension cultures showed tissue specific expression of anthraquinones, flavonoids and phenolics. The root suspension culture showed 198 % and 204 % and fruit suspension culture showed 146 % and 161 % higher production of anthraquinones and flavonoids respectively than widely used leaf suspension culture whereas the phenolic contents were not differed at high extents in leaf, fruit and root suspension cultures. Also, the subculturing of these cultures exhibited no detrimental effect on production of anthraquinones, flavonoids and phenolics. Thus, the root and fruit suspension cultures can be proved as very efficient systems for the production of medicinally important secondary metabolites i.e. anthraquinones, flavonoids and phenolics on large scale so that these compounds can be used as natural medicines to cure and prevent many diseases.

Keywords: Anthraquinones; Flavonoids; Indian Mulberry; *Morinda citrifolia;* Phenolics; Suspension culture.
Abbreviations: G.R.A.S.: Generally Regarded As Safe; F.D.A.: Food And Drug Administration; U.S.: United States; NAA: α-Naphthalene Acetic Acid; BA: Benzyl Adenine; MS medium: Murashige and Skoog medium; B5: Gamborg's medium.

Introduction

Indian Mulberry (*Morinda citrifolia*), commonly known as Noni, is one of the traditional folk medicinal plants that have been used for over 2000 years in Polynesia. It has been reported to have a broad range of therapeutic effects including antibacterial, antifungal, antiviral, antitumor, antihelmin, analgesic, hypotensive, anti-inflammatory and immune enhancing effects (Wang et al., 2002). Indian Mulberry (*Morinda citrifolia*) has recently gained a great deal of interest by scientists and medical professionals due to the pharmaceutical values this plant offers (Hemwimon et al., 2007). Also this plant and its products have been officially designated as G.R.A.S. by F.D.A. and Department of Agriculture at U.S. Therefore, nowadays Indian Mulberry or Noni products are widely used as health tonics as well as an alternative medicine. Plant cell and tissue cultures have been used as an attractive alternative sources to the whole plant for the production of valuable natural products such as pharmaceuticals and neutraceuticals (Chong et al., 2005) because it produces higher yield of secondary metabolites than the whole plant in less time period. Out of all *in vitro* techniques, suspension cultures are more advantageous to produce important compounds in higher quantity and so they have been widely used since they provide a homogeneous system allowing easy environmental control of the cells and scale up. The anthraquinones, flavonoids and phenolics are the major groups of secondary metabolites which are mostly responsible for all the therapeutic properties of the plant Indian Mulberry (*Morinda citrifolia*). Also, nowadays, it is very essential to explore the natural and more economic secondary metabolites to overcome the toxicity and higher manufacturing cost of synthetic medicines. So, there is a great need to produce these important compounds in *in vitro* on large scale without harvesting the complete plants and independent from the environmental factors by using the suspension cultures for fulfillment of the today's demand of increasing population. Considering all the above mentioned needs, the present study was undertaken to establish the suspension cultures from various explants of Indian Mulberry (*Morinda citrifolia*) and to detect the anthraquinones, flavonoids and phenolics qualitatively and quantitatively in suspension cultures derived from leaf, fruit and root. Simultaneously, the effect of subculturing on production of these three groups was also studied.

Materials and methods

Plant Material

The plants of Indian Mulberry (*Morinda citrifolia*) were collected in month of December from the Vidarbha region of Maharashtra, State of India. The plants were identified and authenticated by the agriculture experts. Also the botanical aspects of the whole plant were studied in detail. The specimen vouchers of plant are deposited in our laboratory for future reference. The major and healthy plant parts i.e., leaves, fruits and roots were selected for the establishment of callus cultures and were surface sterilized by using 10 % mild disinfectant and 0.1 % $HgCl_2$. The small pieces (2-4 mm) of

surface sterilized plant materials were used as explants for further experiments.

Callus Cultures

Calli were induced from the surface sterilized explants of leaves, fruits and roots. Many types of media along with various combinations of growth hormones were used to induce calli. The desirable callus cultures were maintained on Murashige and Skoog (MS) medium supplemented with 20 g/l sucrose as a sole source of carbon, 2 mg/l naphthalene acetic acid (NAA), 1 mg/l benzyl adenine (BA), Kinetin (0.2 mg/l) and 0.25% phytagel. The initial pH of the medium was adjusted to 5.8 before autoclaving. The cultures were incubated at 22 ± 2^0C with 16 hours light and 8 hours dark. The callus cultures were subcultured after every 30 days.

Suspension Cultures

Suspension cultures of Indian Mulberry were developed by inoculating approximately 1-2 gm of callus of leaf, fruit and root into the respective liquid medium. Suspension cultures were maintained on the medium having the same formulations as for the callus cultures except phytagel. The suspension cultures were incubated in orbital shaking incubator (90 rpm) at 24±2° C in dark condition. Subculturing of every suspension culture was made at a regular period of 14 days.

Phytochemical Screening

The powdered samples of leaf, fruit and root suspension cultures of Indian Mulberry (*Morinda citrifolia*) were screened for phytochemical constituents using standard procedures of analysis (Odebiyi and Sofovora, 1978; Banso and Ngbede, 2006; Williamsons et al., 1996).

Qualitative Analysis of Anthraquinones, Phenolics and Flavonoids

The qualitative analysis of major groups of secondary metabolites i.e. anthraquinones, flavonoids and phenolics was done by thin layer chromatography (TLC) technique on preparative silica gel (silica gel-60 and silica gel-254) plates and various chemical tests. In TLC, anthraquinones were analyzed on the solvent system ethyl acetate:methanol:water (100:13.5:10), flavonoids on toluene:ethyl acetate: methanol: formic acid (32:14:12:5) and chloroform:methanol (9:1) and phenolics on butanol:acetic acid:water (4:1:5 and 6:1:2). The identification was done on the basis of colour and Rf values under UV light at 365 nm.

Quantitative Analysis of Anthraquinones, Phenolics and Flavonoids

The experiment was carried out in Erlenmeyer's flask (250 ml) containing 100 ml of cell suspension culture and the subculturing of all suspension cultures was done after every 15 days. The intracellular as well as extracellular anthraquinones, phenolics and flavonoids were quantified from all three types of suspension cultures as follows:

Analysis of Anthraquinones

Indian Mulberry (*Morinda citrifolia*) cells from the suspension cultures of leaf, fruit and root were boiled in 80% aqueous ethanol (1 mg/ml of dry weight) for 45 min. in

Table 1. Components of leaf, fruit and root derived suspension cultures of Indian Mulberry (*Morinda citrifolia*) based on the preliminary phytochemical screening.

Secondary Metabolites	Leaf Suspension Culture	Fruit Suspension Culture	Root Suspension Culture
Alkaloids : Hager's Reagent	+	+	+
Mayer's reagent	—	—	—
Anthraquinones	+	+	+
Flavonoids	+	+	+
Phenolics	+	+	+
Steroids	+	+	+
Tannins	+	+	+

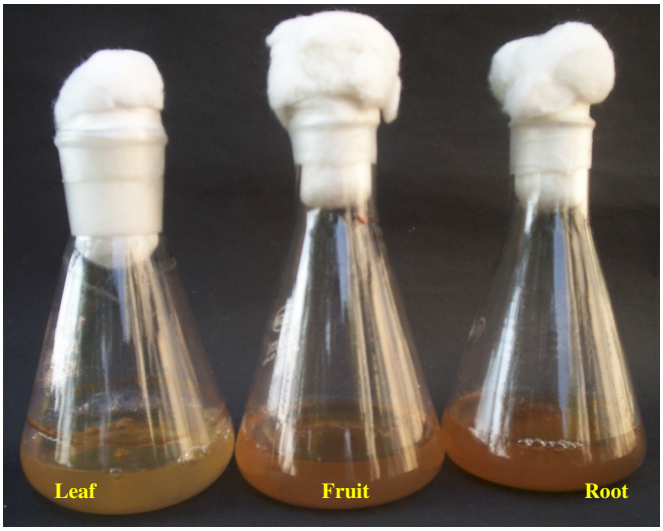

Fig 1. Suspension cultures derived from leaf, fruit and root explants of Indian Mulberry (*Morinda citrifolia*).

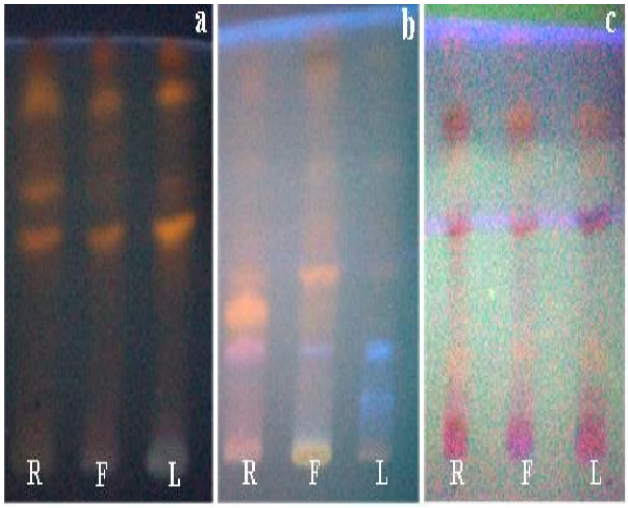

Fig 2. Thin layer chromatographs of leaf, fruit and root cells suspension culture extracts of Indian Mulberry (*Morinda citrifolia*) showing presence of anthraquinones (a), flavonoids (b) and phenolics (c). (L: Leaf suspension culture; F: Fruit suspension culture; R: Root suspension culture).

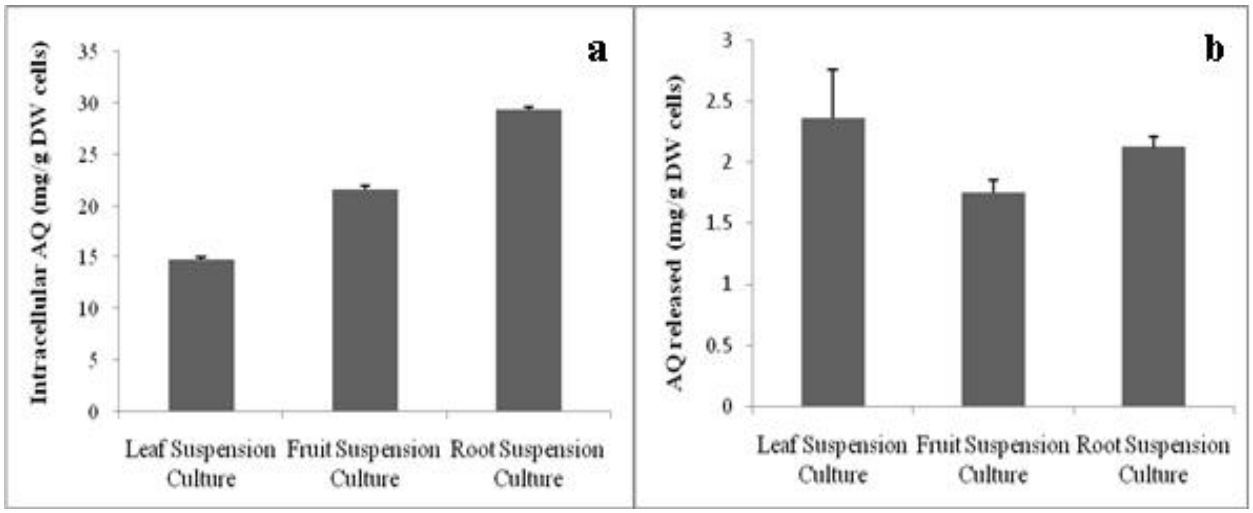

Fig 3. Production of intracellular (a) and extracellular (b) anthraquinones (mg/g dry weight of cells) in leaf, fruit and root suspension cultures of Indian Mulberry (*Morinda citrifolia*).

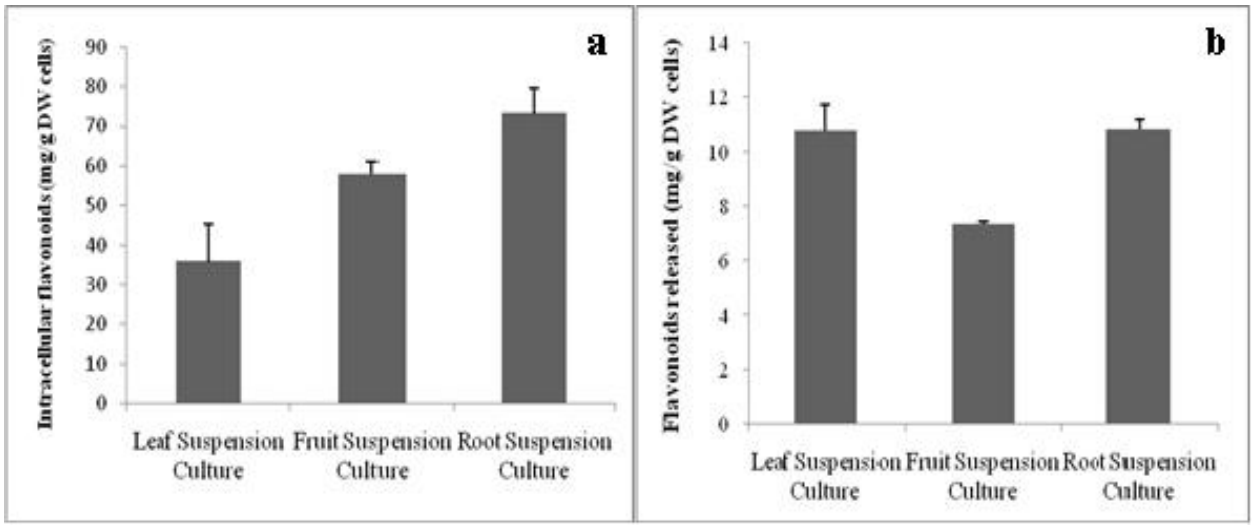

Fig 4. Production of intracellular (a) and extracellular (b) flavonoids (mg/g dry weight of cell) in leaf, fruit and root suspension cultures of Indian Mulberry (*Morinda citrifolia*).

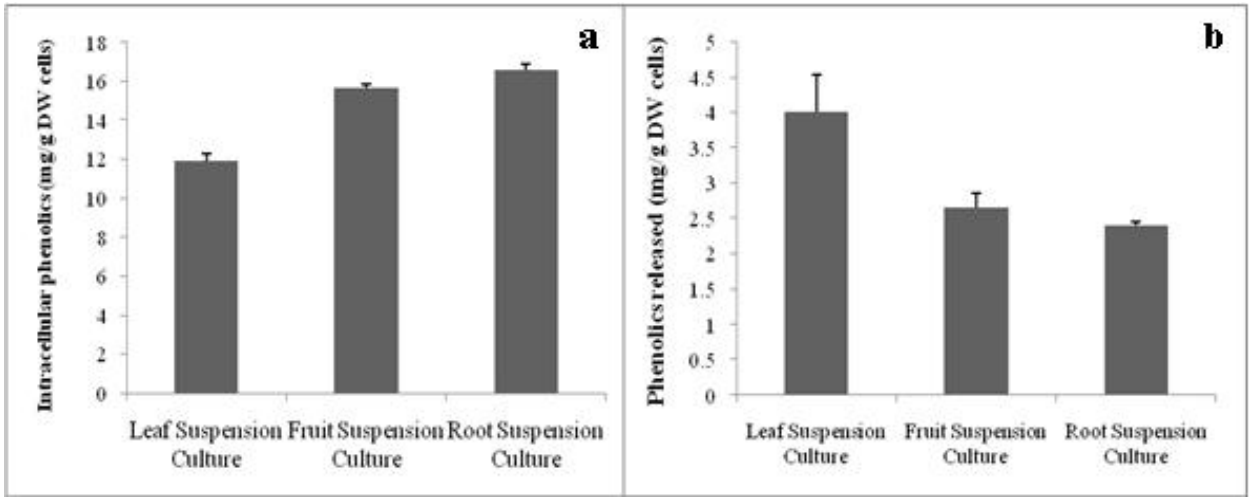

Fig 5 . Production of intracellular (a) and extracellular (b) phenolics (mg/g dry weight of cell) in leaf, fruit and root suspension cultures of Indian Mulberry (*Morinda citrifolia*).

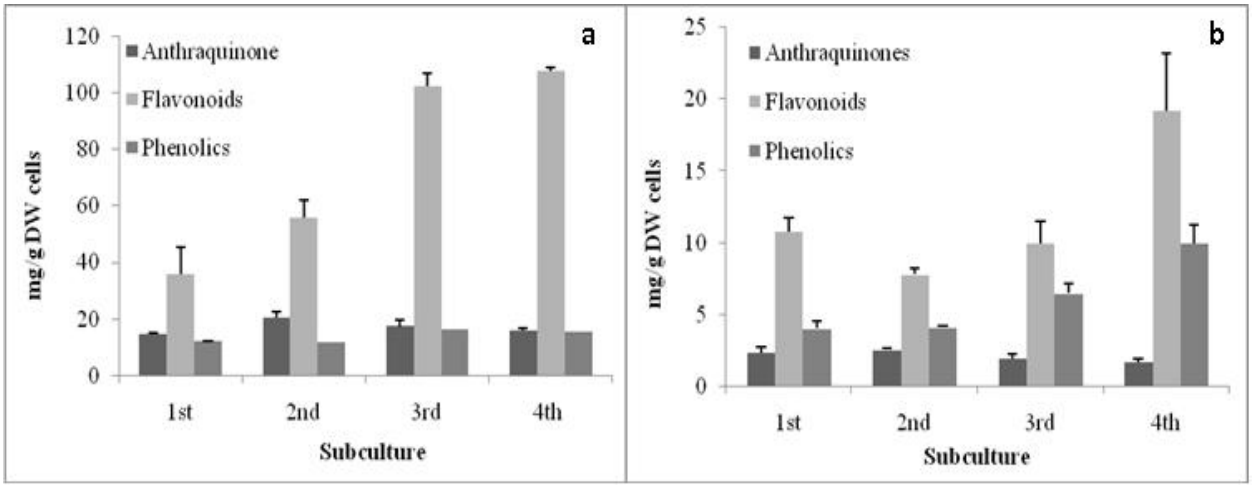

Fig 6. Effect of subculture on intracellular (a) and extracellular (b) production of anthraquinone, flavonoids and phenolics (mg/g of dry cell weight) in cells originated from leaves of Indian Mulberry (*Morinda citrifolia*)

boiling water bath. Then all the samples were centrifuged and the supernatant was collected. More than 99% of the anthraquinone was extracted from the cells by this method (Hagendoorn et al., 1994). The absorption was determined at 434 nm on U.V. Visible spectrophotometer and anthraquinone content was estimated using the molar extinction coefficient of 5,500 for alizarin, considering that differences in the molar extinction coefficients of different anthraquinones did not exceed 5 % (Zenk et al., 1975). The intracellular AQ content was expressed as mg/g of dry weight of cells while extracellular AQ content was expressed as mg/l of culture medium. The leaf, fruit and root suspension cultures revealed the presence of many anthraquinones, out of which 1,2,3 trihydroxyanthraquinone, lucidin, alizarin, ruberythric acid, 2-hydroxyanthraquinone and xanthopurpurin were identified by HPLC analysis (Data not shown).

Analysis of Total Phenolics

The total phenolic contents of leaf, fruit and root derived suspension cultures of Indian Mulberry (*Morinda citrifolia*) were determined with Folin-Ciocalteu reagent using the method of Spanos and Wrolstad (1990). Total phenolics were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW). Similarly, the extracellular phenolics were measured in cell free medium extracted with Mean differences were considered significant at the $P < 0.005$ level.

Results and discussion

Establishment of Callus Cultures

The major parts of Indian Mulberry (*Morinda citrifolia*) plant such as leaf, fruit and root were selected to develop suspension cultures for the detection and enhancement of anthraquinones, flavonoids and phenolics production. To establish the suspension cultures, callus cultures were developed from leaf, fruit and root explants of *Morinda citrifolia*. MS and B5 media with different concentrations and combinations of plant growth regulators were used to induce the callus culture from leaf explant of Indian Mulberry (*Morinda citrifolia*). Out of these various combinations of media, the MS medium formulated with 2 mg/l NAA, 1 mg/l BA and 0.2 mg/l Kinetin along with 2% sucrose and 0.25 % phytagel was found to be more effective to induce the callus. 80% ethanol and were expressed as milligrams of gallic acid equivalent per liter of medium (mg GAE/liter of medium). The cell cultures showed presence of many phenolic acids such as gallic acid, chlorogenic acid, ferulic acid and cinnamic acid in HPLC analysis (Data not shown).

Analysis of Total Flavonoids

Total flavonoid contents were measured by the aluminium chloride colorimetric assay as described by Zhishen et al. (1999). Total flavonoid contents of all suspension cultures of Indian Mulberry (*Morinda citrifolia*) were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g of DW). Total extracellular flavonoids were measured like phenolics and expressed as milligrams of quercetin equivalent per liter of medium (mg QE/liter of medium). The leaf, fruit and root suspension cultures showed large number of flavonoids in HPLC analysis but only quercetin and rutin were identified (Data not shown).

Statistical Analysis

All experiments were conducted in triplicate and statistical analysis was done. The data were presented as mean ± SD.

This medium showed 100% callus induction rate within 12 to 15 days. 20 replicates were used per treatment. Same medium was used to induce the callus cultures from fruit and root explants of Indian Mulberry (*Morinda citrifolia*). The calli induced from leaf, fruit and root explants of this plant were very friable, healthy, well growing and orange yellow in color but the yellow color turned into brown color when calli grew older. The callus cultures of all explants were maintained for 4-5 months by monthly subculturing and then used for the establishment of suspension cultures.

Establishment of Suspension Cultures

The cell suspension cultures of leaf, fruit and root were established by transferring the respective callus cultures to the liquid MS medium having the same formulation like callus culture except phytagel. After the inoculation of callus cells into liquid medium, it took about 10-12 months after several subcultures for the suspended cells to become more homogeneous and stable. The freely suspended cells as well

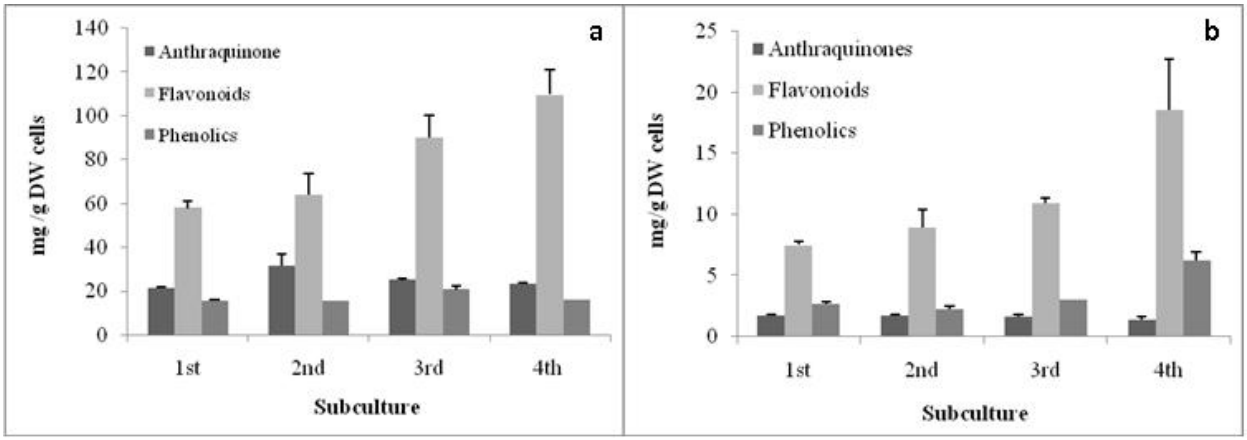

Fig 7. Effect of subculture on intracellular (a) and extracellular (b) production of anthraquinone, flavonoids and phenolics (mg/g of dry cell weight) in cells originated from fruit of Indian Mulberry (*Morinda citrifolia*)

as medium were yellow to orange in color. It might be due to the intracellular and extracellular production of anthraquinones and phenolics as Schripsema et al. (1999) demonstrated that this color has been attributed to anthraquinones and phenolics formation. The leaf suspension culture showed yellowish color whereas fruit and root suspension cultures showed orange color (Fig 1). Subculturing of every suspension culture was made at a regular period of 14 days.

Phytochemical Screening

The preliminary phytochemical screening was done by some standard chemical tests. This revealed the presence of alkaloids, anthraquinones, flavonoids, phenolics, tannins and steroids in leaf, fruit and root cell suspension cultures of Indian Mulberry (*Morinda citrifolia*). The result of the phytochemical screening is presented in Table 1.

Qualitative Analysis of Anthraquinones, Flavonoids and Phenolics

The qualitative analysis of anthraquinones, flavonoids and phenolics was done by Thin Layer Chromatography (TLC). In TLC, the qualitative analysis of anthraquinones, flavonoids and phenolics was done on preparative silica gel plates using specific solvent systems for each secondary metabolite's group. When the alcoholic extracts of leaf, fruit and root derived cell suspension cultures of Indian Mulberry (*Morinda citrifolia*) were subjected to the solvent system ethyl acetate:methanol:water (100:13.5:10), which is specific for anthraquinones (Wagner et al., 1993; Nandhasri et al., 2005), each sample showed many orange colored bands on preparative silica gel plates under ultraviolet light indicating the presence of various anthraquinone derivatives (Fig 2). Similarly, the alcoholic extracts of all suspension cultures were applied to silica gel plates and developed in ethyl acetate:formic acid:acetic acid:water (100:11:11:27) (Nandhasri et al., 2005) and in butanol:acetic acid:water (4:1:5 and 6:1:2) for detection of flavonoids and phenolics respectively. The samples showed orange, pink and blue bands on flavonoids specific solvent system whereas pinkish orange bands on phenolics specific solvent system indicating their presence in tested samples. Thus, the leaf, fruit and root suspension culture extracts showed presence of all three medicinally important groups of secondary metabolites i.e., anthraquinones, flavonoids as well as phenolics in the form of various bands. The root suspension culture extract showed greater intensity in the color of bands than fruit and leaf suspension culture extracts of Indian Mulberry (*Morinda citrifolia*). Also, the root and fruit suspension culture extract showed higher number of bands than leaf suspension culture extracts in case of anthraquinones and flavonoids whereas same number of bands were observed in all three suspension culture extracts in case of phenolics.

Production of Anthraquinones, Flavonoids and Phenolics in Leaf, Fruit and Root Suspension Cultures of Indian Mulberry (Morinda citrifolia)

The cell suspension cultures were established from the leaf, fruit and root explants of Indian Mulberry (*Morinda citrifolia*) for the production of anthraquinones, flavonoids and phenolics which was evaluated by using various standard spectrophotometric methods. The quantitative results of anthraquinones, flavonoids and phenolics are described as follows:

Anthraquinones

The leaf, fruit and root suspension cultures of Indian Mulberry (*Morinda citrifolia*) showed variable range in intracellular as well as extracellular anthraquinone production. The leaf suspension culture produced 14.73 ± 0.25 mg/g of intracellular anthraquinones whereas the fruit and root suspension cultures produced 21.55 ± 0.38 mg/g and 29.27 ± 0.25 mg/g of intracellular anthraquinones respectively. Thus, the root suspension culture showed 198 % and fruit suspension culture showed 146 % higher production of intracellular anthraquinones than leaf suspension culture of Indian Mulberry (*Morinda citrifolia*). However, the anthraquinone contents which were released into the medium by the cells, i.e. extracellular anthraquinones, were found to be similar in leaf (2.36 ± 0.39 mg/g) and root (2.12 ± 0.08) suspension cultures. Slightly low secretion of extracellularly released anthraquinone content was found in fruit suspension culture (1.75 ± 0.10 mg/g). The results are depicted in Fig 3a and 3b. The results of anthraquinones production in root suspension culture are in agreement of Kamiya et al. (2010) who suggested that the noni roots contain higher amount of anthraquinones.

Flavonoids

Like anthraquinones, the leaf, fruit and root suspension cultures of Indian Mulberry (*Morinda citrifolia*) have shown

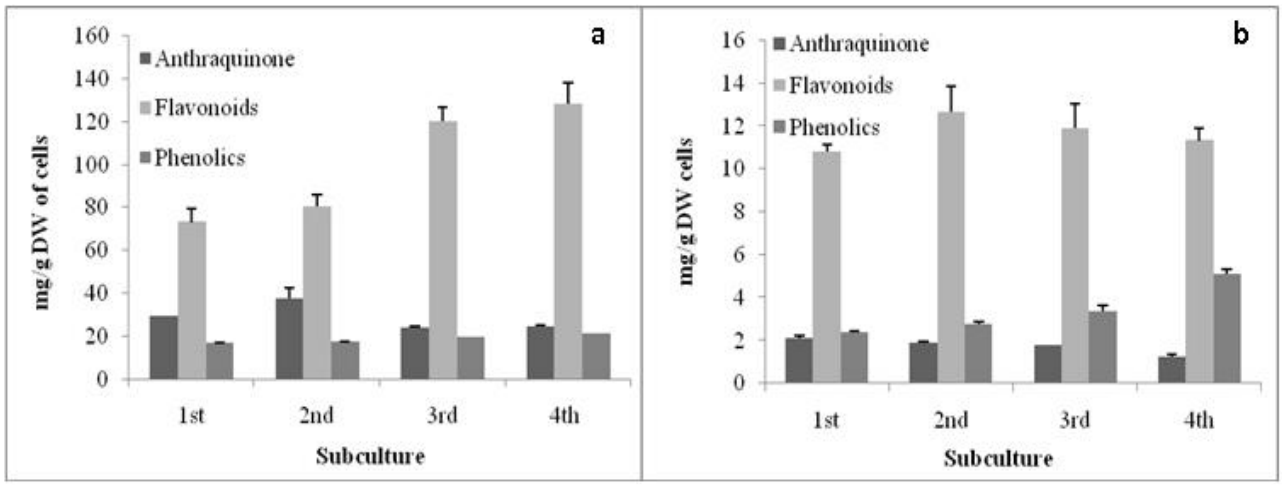

Fig 8. Effect of subculture on intracellular (a) and extracellular (b) production of anthraquinone, flavonoids and phenolics (mg/g of dry cell weight) in cells originated from root explant of Indian Mulberry (*Morinda citrifolia*).

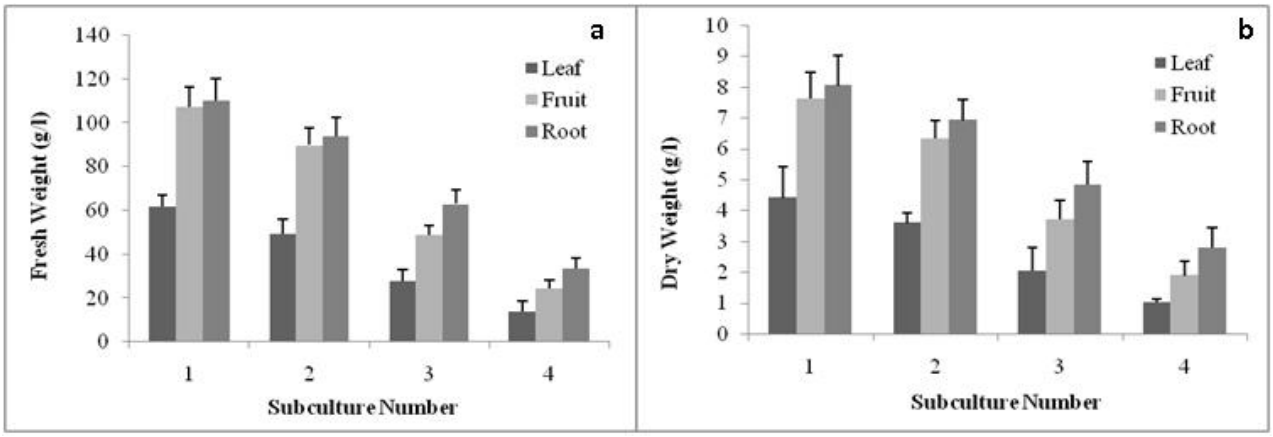

Fig 9 . Effect of subculture on fresh weight (a) and dry weight (b) of cells (g/l) in leaf, fruit and root suspension cultures of Indian Mulberry (*Morinda citrifolia*).

similar pattern in flavonoids production. The root suspension culture showed highest production of flavonoids whereas leaf suspension culture showed lowest production in intracellular flavonoids (Fig 4a). The leaf suspension culture showed 35.8 ± 9.61 mg/g of intracellular flavonoids production. The fruit suspension culture produced 57.9 ± 3.25 mg/g of intracellular flavonoids which were 161% higher than leaf suspension culture. However, root suspension culture produced 73.2 ± 6.22 mg/g of intracellular flavonoids which were 204 % higher than leaf suspension culture. The leaf suspension culture and root suspension culture showed 10.74 ± 0.97 and 10.79 ± 0.36 mg flavonoids secretion per gram of dry cell weight respectively whereas the fruit suspension culture showed slightly reduced production of extracellular flavonoids which was 7.34 ± 0.08 mg/g of dry cell weight (Fig 4b). The intact fruits of Indian Mulberry (*Morinda citrifolia*) contain 5 to 12.3 mg/g of flavonoids (Deng et al., 2008) whereas the leaves produced 1.6 to 3.72 mg/g of flavonoids (Ramamoorthy and Bono, 2007). Thus, the cell suspension cultures of Indian Mulberry (*Morinda citrifolia*) showed very high amount of flavonoids production as compared to its *in vivo* parts.

Phenolics

In leaf, fruit and root suspension cultures of Indian Mulberry (*Morinda citrifolia*), no marked variability was observed in intracellular phenolics production. The root and fruit suspension cultures showed slight increase in intracellular phenolics production than leaf suspension culture (Fig 5a). The leaf, fruit and root suspension cultures produced 11.9 ± 0.42 mg/g, 15.65 ± 0.21 mg/g and 16.60 ± 0.28 mg/g of intracellular phenolics respectively. Similarly, the extracellular phenolics were obtained as 4.01 ± 0.51 mg/g, 2.66 ± 0.18 mg/g and 2.40 ± 0.04 mg/g in leaf, fruit and root suspension cultures respectively (Fig 5b). Thus, the cells of leaf suspension culture showed higher release of phenolics into the medium than fruit and root suspension cultures. But, the production of phenolics in cell suspension cultures of Indian Mulberry (*Morinda citrifolia*) was found to be almost greater than the *in vivo* parts of this plant when compared to the results of Krishnaiah et al. (2007) which reported the total phenolics contents in intact leaf, fruit and root parts of *Morinda citrifolia* which were 9.06 mg/g, 8.73 mg/g and 14.88 mg/g respectively.

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Effect of Subculture on Production of Anthraquinones, Flavonoids and Phenolics

In cell suspension cultures, the cells utilize the medium components for their growth due to which nutrient depletion occurs and the growth of cells ceases. Therefore, there is a need of subculturing of these suspension cultures after a specific time period for their maintenance. By considering

this need, the effect of subculturing of suspension cultures on intracellular as well as extracellular production of anthraquinones, flavonoids and phenolics was studied by using various spectrophotometric assays. In leaf suspension culture, the highest level of intracellular anthraquinone concentration (20.45 mg/g) was obtained after two subcultures showing significant statistical difference with other subcultures but then gradually decreased whereas the intracellular flavonoids were gradually increased from 35.8 mg/g to 107.6 mg/g dry cell weight during the four cycles of subculturing. The highest intracellular phenolic contents of 16.20 mg/g were obtained at 3rd subculture (Fig 6a). Similarly, the extracellular anthraquinone concentration was slightly decreased from 1st subculture (2.36 mg/g) to 4th subculture (1.68 mg/g) whereas the extracellular flavonoids were remain maintained up to 3rdsubculture but increased at 4th subculture up to 19.17 mg/g of dry cell weight but the extracellular phenolics showed increase from 1st to 4th subculturing i.e. from 4.01 to 9.94 mg/g of dry cell weight (Fig 6b). In fruit suspension culture, the highest intracellular anthraquinones (21.55 mg/g) were obtained at 3rd subculture and remained near about constant at 1st, 2nd and 4th subculturing whereas the intracellular flavonoids were increased from 57.9 to 109.7 mg/g of cell dry weight during 1st to 4th subcultures. However, the intracellular phenolic contents were increased at 3rd subculture up to 20.6 mg/g as compared to others (Fig 7a). The extracellular anthraquinones were slightly decreased from 1.75 to 1.35 mg/g but the extracellular flavonoids were gradually increased from 7.50 to 18.55 mg/g during 1st to 4th subculturing of fruit suspension culture. The highest phenolics were obtained at 4th subculture up to 6.24 mg/g of dry cell weight which was 2.5 times more than the phenolic contents of 1st subculture (Fig 7b). In root derived cell suspension culture, the highest intracellular anthraquinones (37.73 mg/g) were obtained at 2nd subculture as observed in leaf and fruit suspension cultures but remained near about same at 1st, 3rd and 4th subculture cycles whereas the flavonoids were gradually increased from 73.2 to 128.6 mg/g during 1st to 4th subcultures. However, root suspension culture showed slight increase in intracellular phenolic contents after every subculture. The phenolics were increased from 16.6 to 21.15 mg/g of dry cell weight during 1st to 4th cycles of subculturing (Fig 8a). The extracellular production of anthraquinones and flavonoids was slightly decreased whereas the phenolics content was slightly increased after every subculture (Fig 8b). The extracellular anthraquinones were decreased from 2.12 to 1.26 mg/g and flavonoids were decreased from 12.65 to 11.31 mg/g but the phenolics were increased from 2.4 to 5.10 mg/g of dry cell weight during subculturing (Fig 8b). Error bar represents mean S.D. (n = 3) in all figures. Fresh and dry weight were found to be the highest at first subculture of leaf, fruit and root suspension cultures but it was gradually decreased after every subculturing of suspension cultures (Fig 9a and 9b). The production of major groups of secondary metabolites increased with decreasing the biomass of suspension cultures. These findings are in agreement with the results reported by Bulgakov et al. (2002) which showed that total anthraquinone production in *Rubia cordifolia* transgenic cultures was higher when a decrease of fresh biomass accumulation of the transgenic cultures was obtained. The leaf, fruit and root derived suspension cultures, which were derived from the various parts of a single plant, showed tissue specific expression of anthraquinones, flavonoids and phenolics. This may be due to the concept of epigenetics which is based on the interaction between genes and their products. The epigenetics plays an important role in cellular differentiation allowing cell types to have specific characteristics despite sharing the same DNA sequence due to either histone modification or DNA methylation. The binding of epigenetic factors to histone tails alters the extent to which DNA is wrapped around histones and the availability of genes in the DNA gets activated. Also, the chromosomes are inherited in cell cultures from their original tissues by methylation of cytosine bases in DNA. Due to these reasons, the cells might be remained differentiated and not converted into dedifferentiated cells. Thus, the results shows that the *in vitro* systems of cell suspension cultures of Indian Mulberry (*Morinda citrifolia*) are very efficient for the production of medicinally important groups of secondary metabolites i.e. anthraquinones, flavonoids and phenolics intracellularly as well as extracellularly. They can be proved as promising alternatives for the commercial production of these compounds for long time, as they are easy to maintain without any loss in secondary metabolites production. The leaf, fruit and root suspension cultures of *Morinda citrifolia* produced higher quantity of anthraquinones, flavonoids and phenolics than the intact plant parts. Also, the subculturing of these cultures showed no inhibitory effect on production of anthraquinones, flavonoids and phenolics which are produced intracellularly as well as extracellularly. Thus, the root and fruit suspension cultures can be proved as very efficient systems for the production of medicinally important secondary metabolites, anthraquinones, flavonoids and phenolics on large scale so that these compounds can be used as natural medicines to cure and prevent many diseases as they produced higher quantity of secondary metabolites than leaf suspension culture of Indian Mulberry (*Morinda citrifolia*).

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