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PHYTOCHEMICAL INVESTIGATIONS OF *DERRIS INDICA* (LAM) BENETTE *IN VIVO* AND *IN VITRO*

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Various plant parts and callus cultures raised from seeds of *Derris indica* (Lam) Benette on MS medium later transferred on RT medium were collected separately and analysed for their carbohydrates, proteins, lipids, ascorbic acid, pigments and phenols. The callus sample showed a significant high amount of starch as compared to other plant parts. Seeds gave higher recovery of carbohydrates, proteins, phenols, lipids and ascorbic acid than callus and other plant parts.

Keywords: Callus, *Derris indica,* Growth index, Tissue culture.

Introduction

Derris indica (Lam) Benette (previously known as *Pongamia pinnata* Linn. and *P.glabra* Vent.) is commonly known as *karanja,* Botanical drug. Charak named it *Chirabilwa* in his fifty Mahakasya under Dashemani Lekhaniyani as anti-inflammatory. Besides this a number of medicinal properties are also attributed in treatment of paromychia[1], wound healing activity[2], psoriasis and psychoromatic disorders[3], anticonvulsant and analgesic[4], antifungal[5], antibacterial[6], CVS and CNS[7], nematicidal[8], hypoglycaemic[9] and agglutinating effect[10]. However, there is no report on its primary phytochemical constituents from plant and its tissue culture.

The present investigation was therefore undertaken for evaluation of total soluble sugars, starch, ascorbic acid, proteins, phenols, chlorophylls, carotenoids, lipid contents from plant parts (seeds, leaves, stem) and callus cultures of *D. indica*

Material and Methods

Plants of *D. indica* were collected from the fields of Maharani College, Jaipur in the month of Jan-Feb. The plant was identified and specimen deposited in the Rajasthan University herbarium. Various plant parts were separated, washed, kept at 100°C in an oven to inactivate the enzymes for 10 min then at 60°C to achieve constant weight and various dried plant parts were powdered. The estimation of ascorbic acid and pigment was carried out on fresh weight basis.

Tissue cultures: The seedlings from the seeds of *D. indica* on Murashige and Skoog's medium[11] were transferred to *RT medium*[12] supplemented with 4 ppm of 2,4-D and 1% agar and maintained for 6 months by frequent subculturings of 6-8 weeks time interval at 26±1°C, 55% relative humidity and diffused light (300 lux). The calli were harvested at different time intervals (2,4,6,8 weeks), dried separately at 100°C for 15 min to inactivate the enzymes followed by drying at 60°C till constant weight was achieved and growth indices (GI) were calculated.

$$GI = \frac{\text{Final dry wt - Initial dry wt}}{\text{Initial dry wt}}$$

Five such replicates were examined and mean value was taken.

Carbohydrates

A. Total soluble sugars

Extraction : Each of the experimental plant parts and callus material (50 mg) was homogenized in a mortar and pestle with 20 ml of 80% ethanol separately and left overnight. Each sample was centrifuged at 1200 rpm for 15 min, the supernatants were collected separately and concentrated on a water bath using method of Loomis and Shull[13]. Distilled water was added to make up the volume upto 50 ml and processed further for quantitative analysis.

B.Starch

Extraction : The residual mass obtained after extraction of total soluble sugars of each of the test samples was suspended in 1.0 ml of 52% perchloric acid[14]. Later 2.5 ml of water was added in each sample and the mixture was shaken vigorously for 5 min.

Quantitative Estimation : One ml aliquots of each sample was used for the estimation of carbohydrates using the method of Dubois *et al.*[15]. A standard regression curve of standard sugar (glucose) was prepared. A stock solution of glucose 100 g/ml was prepared in distilled water. From this solution, 0.1 to 0.8 ml was pipetted into eight separate test tubes and volume was made upto 1 ml with distilled water. These tubes kept in an ice chest, 1 ml of 5% phenol was added in each tube and shaken gently. Five ml of conc. sulphuric acid was poured so that the steam hits the liquid and tubes were gently shaken during the addition of the acid. Finally the mixture was allowed to stand on a water bath at 26-30°C for 20 min. The characteristic yellow orange colour was developed. The optical density was measured at 490 nm using spectrometer (Carl Zeiss, Jena DDR, VSU 2 P), after setting for 100 % transmission against the blank (distilled water). Standard regression curve was computed between the known concentrations of glucose and their respectiveoptical density, which followed the Beer's law.

All the samples were analysed in the same way as described above and contents of the total soluble sugars and starch were calculated by computing optical density of each of the samples with standard curve.

Ascorbic acid

Extraction : The various test samples (fresh wt. basis) were homogenized with 10 ml of acetate buffer in mortar pestle separately[16]. The homogenized samples centrifuged for 20 min to each of the supernatants (1 ml), 4 ml of 4 % trichloroacetic acid (TCA) was added and left over night. This was then subjected to centrifugation. The supernatant was collected separately, to this 1 ml of colour reagent (prepared by mixing 90 ml of 2.2 % dinitrophenyl-hydrazine in 10 N sulphuric acid, 5 ml of 5% thiourea and 5 ml of 0.6% copper sulphate solution) was added. Mixture so obtained was incubated at 57°C for 45 min, cooled, 7 ml of 65% sulphuric acid was added and cooled again.

Quantitative Estimation : A stock solution of standard ascorbic acid (10 mg/

100 ml) was prepared. Various concentrations ranging from 0.01 to 0.09 mg/ml water were prepared from the stock solution. To this 4 ml of 4% TCA was added, left overnight and centrifuged. To the supernatants 1 ml of colour reagent (prepared as described above) was added and the mixture was incubated at 57°C for 45 min.It was cooled, 7 ml of sulphuric acid was added and cooled again. Five replicates of each concentration was read at 540 nm (against blank). The average values were plotted against the respective concentrations to compute a regression curve. The test samples were processed as stated above and the optical densities computed with the standard regression curve.

Proteins

Extraction : The test samples (50 mg) were separately homogenized in 10 ml of cold 10% trichloroacetic acid (TCA) for 30 min and kept at 4°C for 24 h. These mixtures were centrifuged separately and supernatants were discarded. Each of the residue was resuspended in 10 ml of 5% TCA and heated at 80°C on a water bath for 30 min. The samples were cooled, centrifuged and the supernatants of each were discarded. The residue was then washed with distilled water, dissolved in 10 ml of 1N NaOH and left overnight at room temperature[17].

Quantitative Estimation : Each of the above samples (1 ml) was taken and the total protein content was estimated using the spectrophotometric method of Lowry *et al.*[18]. A regression curve of the standard proteins (Bovine albumin) was prepared. A stock solution of Bovine albumin (Sigma Chem. Co., St. Louis, USA) was prepared in 1N NaOH (1 mg/ml). Eight concentrations (ranging from 0.1 to 0.8 mg/ml were separately measured in test tubes and distilled water was added to make up alkaline solution (Prepared by mixing 50 ml of 2% Na_2CO_3 in 0.1 N NaOH and 1 ml of 0.5% $CuSO_4$. 5 H_2O in 1 % Sodium potassium tartarate) was added and kept at room temperature for 10 min. In each sample 0.5 ml of Folin-Ciocalteau reagent (commercially available reagent was diluted with equal volume of distilled water just before use) was added rapidly with immediate shaking and optical density of each sample measured after 30 min at 750 nm using spectrophotometer against the blank[18]. Five replicates of each concentration were taken and the average value was plotted against their respective concentrations to compute a regression curve.

All the test samples were processed in the same manner and the concentration (total protein content) in each sample was calculated by referring the optical densities of each samples with standard curve. Five replicates were taken in each case and mean value was calculated.

Chlorophylls and Carotenoids

Extraction : Fresh stem and leaves (1 gm each) of the experimental plant were used for the extraction. They were homogenized with 40 ml of 80% acetone[19]. A pinch of sodium bicarbonate was added to each of the homogenates during maceration to prevent the pheophytin formation. Dim light conditions were used during the process to avoid photobleaching[20]. Each sample was centrifuged and supernatants kept separately.

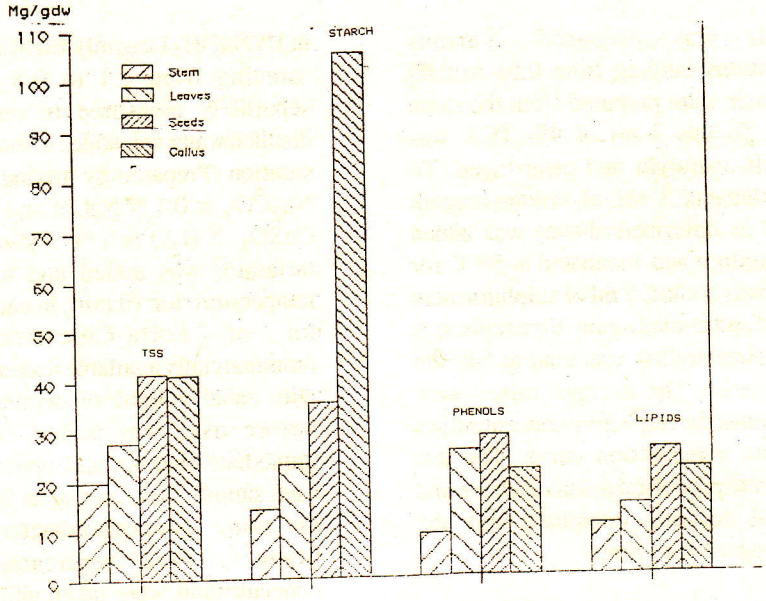

Fig. 1 Total levels of TSS (Total soluble sugars), Starch, Phenols, and Lipids in stem, leaves, seeds and callus of *D. indica.*

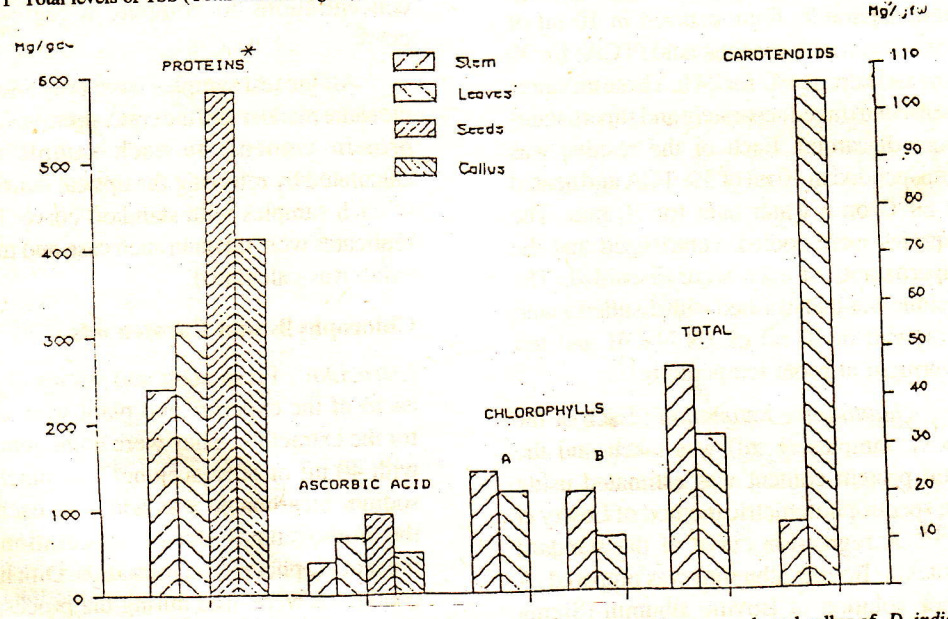

Fig. 2. Total levels of protains, ascorbic acid, chlorophylls and carotenoids in stem, leaves, seeds and callus of *D. indica* : * on dry weight basis.

The residue was macerated with acetone (5 ml) followed by centrifugation until each sample was devoid of chlorophyll. Each of the supernatants were pooled separately and volune was made upto 100 ml with 80 % acetone.Here E^{car} 480 = increase in absorbance at 480 nm due to the carotenoids; E^{Car} 480 = extinction at 480 nm; E_{645} = extinction at 645 nm and E^{Car}_{663} = extinction at 663 nm.

Lipids

Extraction and Quantitation : The test samples were dried, powdered and 100 mg was macerated with 10 ml distilled water, transferred to a conical flask containing 30 ml of chloroform and methanol (2.1; V/V)[21]. The mixture was thoroughly mixed and left overnight at room temperature in dark for complete extraction. Later, 20 ml of chloroform mixed with 2 ml of water were added and centrifuged. Two layers separated, the lower layer of chloroform, which contained all the lipids was carefully collected in the pre weighed glass voils and the coloured aqueous layer of methanol which contained all the water soluble substances and thick pasty outer face layer were discarded in each test sample. The chloroform layers were evaporated to dryness and weighed.Each treatment was replicated thrice and their mean values calculated.

Phenols

Extraction : The deprotenized test materials (200 mg) were macerated with 10 ml of 80% ethanol for 2 h and left overnight at room temperature. The mixtures were centrifuged and the supernatants were collected separately and maintained upto 40 ml by adding 80% ethanol.

Quantitative Estimation : Total Phenol content in each sample was estimated by the spectrophotometric method[22]. It included the preparation of a regression curve of standard phenol (caffeic acid). A stock solution of caffeic acid was prepared by mixing 40 mg of Standard phenol in 1 ml of 80% ethanol. Eight concentrations ranging from 0.1 to 0.8 ml were prepared in test tubes and volume was raised to 1ml of 80% ethanol. In each test tube, 1 ml of Folin-Ciocalteau reagent (commercially available reagent was diluted by distilled water in 1: 2 ratio just before use), 2 ml of 20% sodium carbonate solution was added, shaken thoroughly placed in boiling water for 1 min and cooled under running water. These reaction mixtures were diluted to 25 ml by adding distilled water and the optical density was read at 750 nm against a blank[22]. The optical density of each sample was plotted against the respective concentration of total phenols. The concentrations in the test samples were calculated by referring the respective optical density of test samples against standard curve of caffeic acid.

Results and Discussion

The seeds of *D. indica* took 15 to 20 days to germinate. The seedlings germinated on MS medium[11] were transferred to RTmedium[12] which showed better callus growth containing 4 ppm of 2,4-D. The seedling on MS medium showed organogenesis and lot of root formation. The radical portion of the seedlings initiated the callus formation which is creamish white and fragile.

The growth index was minimum in the 2 week old tissue (0.13) which increased in 4 weeks(0.17) and reached to maximum in 6 weeks (0.21) which later declined in 8 weeks (0.19) depicting a sigmoid curve.

Among the plant parts seeds showed the maximum amount of all metabolites except photosynthetic pigments. Among the

photosynthetic pigments, the total chlorophylls and carotenoids content was found to be maximum in leaves (chlorophylls 3.277 mg/gfw; carotenoids 1.0645 mg/gfw). Individually, chlorophyll-a (2.187 mg/gfw) was more than chlorophyll-b(1.093 mg/gfw) in the leaves.

The amount of total sugars was more in seeds and tissue 41.33 mg/gdw and 40.96 mg/gdw respectively and minimum in stem (20 mg/gdw). The maximum levels of starch were observed in callus (105.33 mg/gdw) and minimum in stem (14.2 mg/gdw) (Fig.1).

Niranjan and Katiyar[23] reported a variation in crude protein content of certain leguminous plants (2.80 to 3.10 %) but seeds of *D. indica* showed sufficiently significant protein content (5.84%) (Fig.2).

The lipid content of *D. indica* showed a closed similarity on total lipids range from (1.033 - 2.5%) in various plant parts. A report on phospho lipid composition of *karanja* seeds contained 2.9% lipids[24] which is also in closed proximation with the present study (Fig.1).

The phenol content of D. *indica* in the present study ranged from 1.8 to 2.8%. However, Kamal and Mangala[25] reported 1.0 to 4.8% phenols in *Indigofera tinctoria* . The higher concentration of phenols in pods of *I. tinctoria* is in corelation with the maximum concentration of phenols in seeds of *D. indica* (Fig.1).

Ascorbic acid is the natural vitamin which is present all over the plant kingdom. In the present investigation however, the ascorbic acid contents were low as 7.4% to highest as 17.5% making it quite rich source of alpha xylo ascorbic acid. Among *Trigonella* species natural source of ascorbic acid in aerial parts was estimated 2.7% by Saleh *et al* [26].

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