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SHORT COMMUNICATION

In vitro callus induction from leaf explants of *Lawsonia inermis* L. used as herbal dye

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ABSTRACT

Fresh henna leaves (Lawsonia inermis L.) crushed or dry henna leaf powder is widely accepted as an herbal dye. Thus callus from leaf explants of Lawsonia inermis L. is a new innovation which requires acceptance as an herbal dye. Callus was initiated in leaf explants (young and old) from field grown plants of Lawsonia inermis L. on MS medium supplemented with different concentrations of 2, 4 - dichlorophenoxy acetic acid (2, 4 - D) (0.1-1.0 mgL^{-1}) used singly or in combination with coconut milk (CM), polyvinyl pyrrolidone (PVP), adenine sulphate (AS) and casein hydrolysate (CH). Leaf explants of Lawsonia inermis L. showed moderate to good amount of callus. The presence of lawsone was confirmed in the callus when compared with standard lawsone by TLC method. Patch test showed light brown (golden) to brown colour. This confirmed the colouring potential of dried callus powder.

KEY WORDS: *Lawsonia inermis L., callus, lawsone, herbal dye*

INTRODUCTION

In the present era Herbal Science has emerged as a major focus for trade as there has been a worldwide acceptance of herbal products. However new innovations and an increased willingness to adopt new techniques are required for wider acceptance of such herbal products globally. Synthetic dyes are easily available and have created a comfortable niche in today's ever-growing market. However, a huge market for herbal products exist which needs attention. Fresh leaves of *Lawsonia inermis* L. (crushed) or dry henna leaf powder is widely accepted as a hair dye of herbal origin. *Lawsonia inermis* L. is also used traditionally for the decorating hands and feet [1]. Lawsone is the chief constituent responsible for the dying properties of the plant. Dried powdered leaves of henna contain about 0.5-1.5% lawsone, traditionally used to produce colour fast orange, red and brown dyes [2]. Therefore a biotechnological approach was made as an attempt to capitalize on the potential of henna as a herbal dye to fulfil the market demand.

MATERIALS AND METHODS

In the present study field grown plants were selected. Authentication of the plant was done (S.H.-1533) at Blatter Herberium, St. Xavier's College, Mumbai. Pre-treated leaf explants (young and old) were inoculated on basal MS medium [3], MS medium fortified singly with 2, 4-D, NAA, BAP, Kinetin and Zeatine (0.1 – 1.0 mg/l) and MS medium fortified with various concentration of 2, 4-D + CM; 2, 4-D + AS; 2, 4-D + PVP; 2, 4-D + CH; 2, 4-D + AS + CM; 2, 4-D + AS + PVP + CM. The cultures were maintained at 25 ± 2 ºC under 16 hr photoperiod from cool white fluorescent lamps.

TLC analysis of callus powder (young and old leaf), field grown plant powder and standard lawsone was done using methanolic extracts. 10 µl test solution and standard lawsone was applied on percolated silica gel $60F_{254}$ TLC plate (E. Merck) of uniform thickness. The solvent system used was (5: 4: 1) Toluene: Ethyl acetate: Acetic acid [4].

Callus powder was soaked in water over night. Callus paste was applied in patches (1″ X 1″) and kept for an hour for the development of colour. White Wistar Rat used for the study was obtained from animal house, Birla College, Kalyan.

RESULTS AND DISCUSSION

Initiation of callus was not observed when leaf explants (young and old) were inoculated on basal MS medium, MS medium fortified singly with various concentration of NAA, BAP, Kinetin, Zeatin, AS, CH and PVP. However, leaf explants (young and old) inoculated on MS medium fortified with 2, 4-D (0.1 – 1.0 mg/l) showed less to moderate amount of callus. Leaf explants (young and old), inoculated on MS medium fortified with 2, 4 – D (0.1 – 1.0 mg/l) and CM (10 – 15 %) showed moderate to good amount of callus. Young leaf explants, inoculated on MS medium fortified with 2, 4 – D (0.5 mg/l), AS (0.5 mg/l), PVP (0.5 mg/l) and CM (10%) moderate to good amount of callus while old leaf explants did not show any response (Table 1).

TLC of methanolic extracts of callus powder (young and old leaf) and field grown plant powder showed presence of lawsone when compared with standard lawsone solution when detected at visibly, at 254 nm and at 366 nm (Plate 1). In *Lawsonia inermis* L. lawsone accumulation is restricted to aerial part of the plant [5]. Lawsone content was found to be highest in the petiole of young leaves whereas older leaves showed lower lawsone content [1].

In the present study the leaf explants (young and old) from field grown plant of *Lawsonia inermis* L. inoculated on MS medium fortified with different concentrations of 2, 4 – D used singly or in combination with AS, PVP and CM showed moderate to good amount of callus. Lawsone was confirmed in the callus when compared with standard lawsone by TLC method. Patch test showed light brown (golden) to brown colour (Plate 2). Thus, confirming the colouring potential of dried callus powder.

Table 1 Effect of plant growth regulators on leaf explants (young and old) inoculated in MS medium for the initiation of callus

MS Medium + PGR(mg/l) + Addenda		Young leaf	Old leaf
2,4-D (mg/l)	0.3	++	+
	0.4	++	+
2, 4-D (mg/l) + CM (%)	0.3+10	+++	+++
	0.4+10	+++	+++
	0.5+10	++	++
	0.5+15	+++	-
	1.0+15	+++	-
2, 4-D + AS (mg/l)	0.5+0.5	++	-
	1.0+0.5	-	+
2, 4-D + AS (mg/l) + CM (%)	0.5+0.5+10	++	-
2, 4-D + AS + PVP (mg/l) + CM (%)	0.5+0.5+0.5+10	++	-

Values are mean of three sets of determinant. Each set containing 10 explants. + = less callus formation; ++ = moderate callus formation; +++ = good callus formation; - = no response

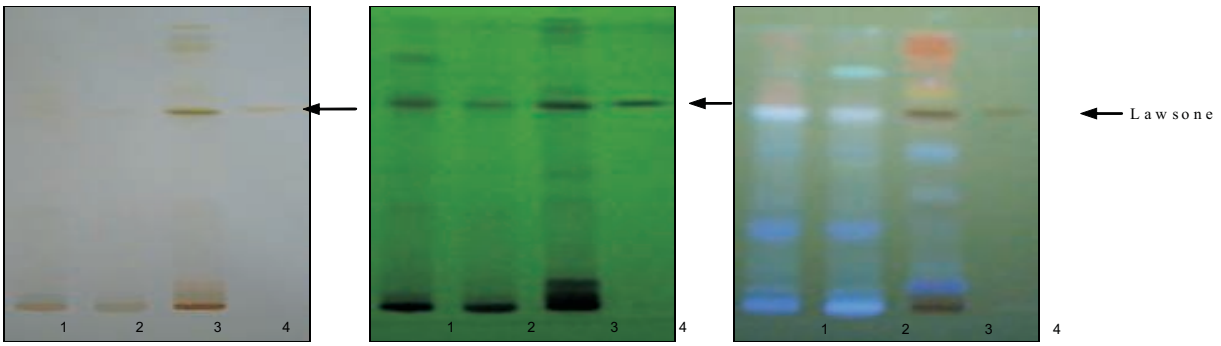

Plate 1 TLC fingerprint of callus powder and leaf powder of *Lawsonia inermis* L. 1 – Young leaf callus powder; 2 – Old leaf callus powder; 3 – Leaf powder of field grown plant; 4 – Standard lawsone

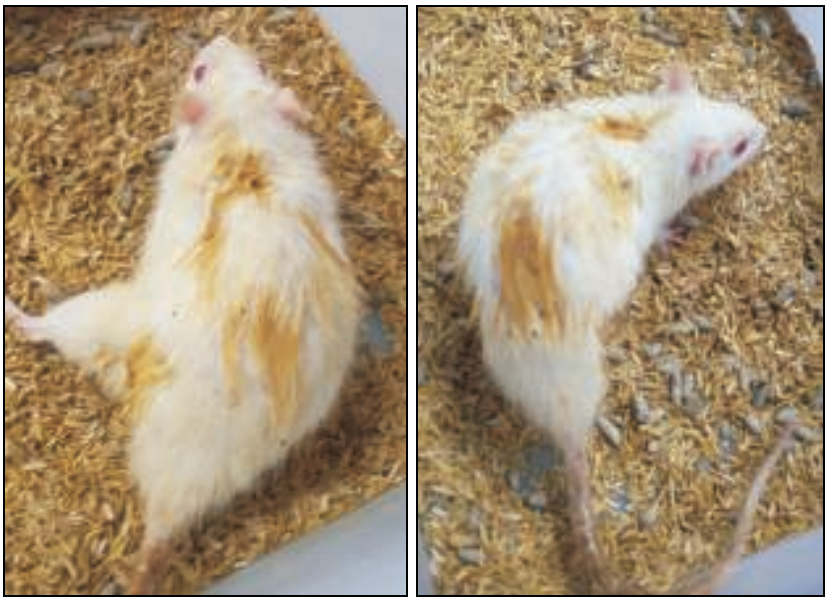

Plate 2 Patch test of callus powder of *Lawsonia inermis* L. Patches of 1″ X 1″ were made on white Wistar rat (in triplicates)

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