end of 2 h up to 4 h, a significant reduction ($P<0.01$) was recorded, when compared to control. Thus, it can be concluded that, on preliminary screening of crude extracts of *Pergularia extensa,* the ethanol (95%) extract and its butanol fraction possessed significant antiinflammatory activity. These results indicate a need for a detailed photochemical investigation of *Pergularia extensa* to identify the active constituents.

ACKNOWLEDGEMENTS

We thank Dr. F. V. Manvi, Principal, K.L.E.S's College of Pharmacy, Belgaum for providing the facilities to carry out the research work. We wish to extend our thanks to Late Shri A. P. Kore, Asst. Professor, Dept. of Biological Sciences, K.L.E.S's College of Pharmacy, Belgaum for authentication of the plant.

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Production of Hypericin from Tissue Culture of *Hypericum perforatum*

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Accepted 24 July 2001
Revised 17 July 2001
Received 6 February 2001

Murashige and Skoog medium supplemented with 2,4-D (2 mg/l) and kinetin (1 mg/l) was found to be suitable for the establishment of callus cultures of *Hypericum perforatum*. The callus upon extraction and analysis by TLC/HPLC revealed the presence of hypericin. The content of hypericin in callus cultures of *H. perforatum* was significantly less than the intact plant. there is no significant effect of media or hormones on bioproduction of hypericin.

H. perforatum (Hypericaceae) is one of the top 15 selling popular herbs in USA and Europe[1]. It is a herbaceous perennial plant widely distributed in Europe, Northern Africa and it is naturalized in the USA. This plant is not described in Ayurvedic system of medicine. It grows widely in the Himalayan regions and in the hills of the central part of India. The local names of this plant are dendhu patta, basant (Hindi) and balasana (Urdu). It contains hypericin (0.05-0.3% w/w on dry weight basis) and hypericin-like substances, notably pseudohypericin, isohypericin, protohypericin, hyperforin and adhyperforin[2]. The hypericum herbal extract was reported to have antiviral, wound healing and hepatoprotective properties. Hypericin was reported to have antidepressant activity[3]. It was recently reported that hyperforin a compound isolated from *Hypericum perforatum* known to inhibit protein Kinase C activity[4].

Plant tissue culture is one of the alternative techniques for the production of phytopharmaceuticals. This technique has been already commercialized for the production of taxol, shikonin and rosmarinic acid[5]. Callus culture of *Hypericum perforatum Var.* augustifolium was established from stem segments of *in vitro* shoot cultures on solid MS medium supplemented with NAA (4.5 μM and kinetin (2.3 μM)[6]. Cardoso[7] reported shoot multiplication and callus induction of *Hypericum*

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brasilience. Optimum callus induction was observed on either MS or B5 medium with 2,4-D (1-2 mg/l). Zdunek[8] reported shoot organ cultures of *H. perforatum* cultured on LS medium with 2,4-D (10.5 μM) and kinetin (10.7 μM) in dark condition. The callus upon extraction yielded, a new paxanthone[9]. However, there is no report on the production of hypericin or hypericin - like substances from *H. perforatum* of Indian origin. In this paper, we report the production of hypericin in the callus cultures of *H. perforatum.* The seeds of *H. perforatum* were procured from the hills of MP and identified in the Department of Botany, Kakatiya University, Warangal, AP, India. The seeds of *Hypericum perforatum* were washed thoroughly with sterile double distilled water and surface sterilized with alcohol 70% v/v for 1 min followed by mercuric chloride 0.1% w/v for 3 min. Then the seeds were washed thoroughly with sterile double distilled water and transferred in to sterile flasks containing sucrose-agar media. The flasks were incubated in a BOD incubator at 25±2° for 2 w. After two weeks, the hypocotyls portions of aseptically germinated seeds were cut into small pieces and transferred onto MS medium containing 2,4-D (2 mg/l) and kinetin (1 mg/l) and incubated at 25±2° in a BOD incubator. The induction of callus was observed on completion of 4 w. The callus cultures were maintained by subculturing on to the same media at a regular interval of 4 w.

The dried and accurately weighed quantity of callus (0.4 g) was homogenized and kept on maceration with methanol (30 ml) for 24 h at room temperature. The residue was extracted with acetone (20 ml) at room temperature and filtered. Both the methanol and acetone filtrates were combined and reduced to dryness under vacuum. The dried material was dissolved in methanol HPLC grade (2 ml) and was used for TLC and HPLC analysis[10].

The methanolic extracts obtained from callus cultures were subjected to TLC analysis using silica gel F UV_{264} aluminum precoated plates (E. Merck). The co-chromatography of methanolic extracts was done with an authentic sample of hypericin using the solvent system of ethyl acetate-formic, acid-glacial, acetic, acid-water (100:11:11:27). Pyridine (10% v/v) in ethanol was used as the detecting system.

The HPLC analysis of the extracts was performed with a Shimadzu^R HPLC (photo diode array detector) SPD-M10A system. Compounds were separated on a Nucleosil^R (25 x 0.4 cm, 5 μ) RP C18 column. The mobile phase used for the analysis was acetonitrile-methanol-water-phosphoric acid (55:20:24:1) with a flow rate of 1 ml/min for 39 min and 0.6 ml/min after 40 min and the detection was at 254 nm. A 10 μl sample of each extract was injected into the injection port with the help of Hamilton syringe and the peaks were recorded. A standard graph was plotted with concentration of hypericin vs peak area. The amount of hypericin present in the extracts was calculated from the standard graph.

TABLE 1: THE CONTENT OF HYPERICIN IN CALLUS CULTURES OF *HYPERICUM PERFORATUM*

Medium	Amount of Hypericine (w/w on dry wt. basis)
Gamborg (B5)	0.030%
Murashige and Skoog	0.023%

*Average of 3 readings.

MS medium supplemented with 2,4-D (2 mg/l) and kinetin (1 mg/l) was found to be suitable for the induction of callus cultures from the explants of *H. perforatum.* A brown colored callus was produced on completion of 4 w of incubation at 25±2° with an exposure to 16 h light. The co-chromatography of extracts obtained from callus cultures with an authentic sample of hypericin showed a pinkish-violet spot similar to that of standard substance indicating the presence of hypericin. The Rf value was found to be 0.8. On HPLC analysis, the extract of cultures has shown a peak similar to the authentic sample and also the retention time 43.8 min revealed the presence of hypericin in the callus cultures of *H. perforatum.* The content of hypericin produced in callus cultures grown on B5 medium was relatively higher than MS medium (Table 1). A study on the influence of different hormones on production of hypericin in callus cultures of *H. perforatum* reflected that both dicamba and 2,4-D have shown almost similar effect on bioproduction of hypericin (Table 2). It was reported that the 2,4-D is sometimes inhibitory to the production of secondary metabolites in cultures[11]. There is no significant effect of auxins or medium on bioproduction of hypericin in callus culture. These results also revealed that the differentiation is not required for *H. perforatum* to produce a secondary metabolite. In conclusion, the callus cultures of *H. perforatum* produced hypericin significantly less than that of intact plant. However, yield improvement strategies

TABLE 2: THE EFFECT OF AUXINS ON BIOPRODUCTION OF HYPERICIN

Hormone	Amount of Hypericine (w/w on dry wt. basis)
Dicamba	0.024%
2,4-D	0.028%

Average of 3 readings, 2,4-D is 2,4-Dichlorophenoxyacetic acid. Dicamba is 3,6-dichloro-o-ansic acid.

have to be developed before commercialization of this technique for the production of hypericin from tissue cultures.

ACKNOWLEDGEMENTS

N. Sulochana Rani is thankful to University Grants Commission for the award of Junior Research Fellowship.

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Spectrophotometric Methods for the Estimation of Satranidazole in Pharmaceutical Formulations

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Accepted 24 July 2001
Revised 17 July 2001
Received 23 October 2000

Two spectrophotometric methods (I and II) in the visible region have been developed for the estimation of satranidazole in bulk drug and pharmaceutical formulations. Methods I and II are based on the reaction of reduced satranidazole with *p*-dimethylaminobenzaldehyde (PDAB) and *p*-dimethylaminocinnamaldehyde (PDACA) in acidic conditions to form orange red and purple coloured chromogens with absorption maxima at 511 nm and 568 nm respectively. The reduction of satranidazole was carried out with zinc granules and 4 N hydrochloric acid at room temperature in ethanol. Beer's law was obeyed in the concentration range of 10-50 µg/ml for both the methods. The results of analysis have been validated statistically and by recovery studies. The methods were found to be simple, rapid, accurate, reproducible and economic. The results are comparable with those obtained using UV spectrophotometric methods in alcohol at 315 nm.

Satranidazole[1], 1-methylsulphonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazoli dinone[2], is one of the large series of nitroimidazoles with a potent antiprotozoal activity[3], against *E. hystolytica, T. vaginalis* and *Giardia.* Satranidazole is chemically different from metronidazole

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