With the development of axenic culture of *P. romagnoliana* on hormone-supplemented MS medium, one major obstacle in its development as a biocontrol agent against purple nutsedge has been overcome. Although mycelial growth and sporulation are better at 20 and 35°C, respectively, the objective of this study was to obtain maximum sporulation in minimum time. For this, 25°C is the optimum temperature. Further studies are needed to standardize the application technology of this axenic culture.

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Polyphenolic components of flowers of *Helichrysum bracteatum*

Helichrysum bracteatum (Asteraceae) is a stout annual herb, naturalized in high altitudes. It is commonly available in the Nilgiris and Kodaikanal hills of south India[1]. The flowers are large, everlasting and appearing in golden yellow, pink and ivory white shades. *Helichrysum* species are rich in secondary metabolites and have good medicinal value[2]. Previous reports reveal that *H. bracteatum* contains lignin and aurones[3,4]. There is no record of isolation of any other constituent. In view of the reported medicinal values of *Helichrysum* species, three differently coloured (golden yellow, pink, ivory white) flowers were examined for their polyphenolics.

Fresh flowers of *H. bracteatum* var. *bracteatum* collected from the Nilgiris hills in south India were refluxed with 90% boiling ethanol and concentrated under reduced pressure. The aqueous crude extract was fractionated into benzene, ether and ethyl acetate solubles. Ether and ethyl acetate soluble fractions tested positive for polyphenolics. These fractions were mixed together, concentrated and kept in an ice chest, where they gave a yellow solid, which on PC and TLC indicated the presence of UV-active compounds Separation was carried out by preparative cellulose TLC[5] with 50% acetic acid as the developing solvent. The separated zones were eluted with hot methanol and the residue recrystallized from MeOH. The compounds characterized[6] and the methods[7] used are given in Table 1.

^{1}H NMR spectral data (400 MHz, $CDCl_3/CD_3OD$, TMS as int. std., δ, ppm) for the two rare flavonoids showed the following characteristic signals: Bractein: 6.71 (s, 2H, H-2′,6′), 6.31 (s, 1H, =CH–), 6 04 (d, J = 2 Hz, 1H, H-7), 5.98 (d, J = 2 Hz, 1H, H-5), 4.66 (d, J = 8 Hz, 1H, anomeric glucose proton), 3.65–3.17 (m, 6H, other glucose protons); cernuoside: 7.22 (d, J = 2 Hz, 1H, H-2′), 6 98 (dd, J = 8,2 Hz, 1H, H-6′), 6.60 (d, J = 8 Hz, 1H, H-5′), 6.42 (s, 1H, =CH–), 6.09 (s, 1H, H-7), 6 02 (s, 1H, H-5), 4.68 (d, J = 8 Hz, 1H, anomeric glucose proton), 3.68–3.19 (m, 6H, other glucose protons). A survey of the distribution flavonoids in Asteraceae reveals the predominance of apigenin and luteolin along with their 7-glucosides[8]. The present finding of luteolin and its 7-glucoside is in further support. The co-occurrence of phenylpropanoid, aurones and flavonoids in *H. bracteatum* is in agreement with the biogenesis of these types of compounds involving common precursors like mevalonic acid, shikimic acid and phenylalanine. The present study revealed that three morphologically similar varieties of *H. bracteatum*, differing only in the colour of the flower, biosynthesize three different flavonoid compounds – aurone glycosides and flavone in golden yellow, aurone glycosides, and flavone O and C-glycosides in pink and flavone-O-glycoside in ivory white and thus correspond to three chemotypes differentiated by chemical constituents. Bractein was screened for anti-cancer and anti-HIV properties with the help of

Table 1. Compounds isolated and characterized from the flowers of *H. bracteatum*

Compound	Golden yellow	Pink	Ivory white	Characterization method
5,7,3′,4′-tetrahydroxyflavone (luteolin)	+	+	+	m p, UV, IR, co-spot with authentic sample
6,3′,4′,5′-tetrahydroxy 4-O-β-D-glucopyranosylaurone (bractein)	+	+	–	m p, UV, IR, ^{1}H NMR, FDMS, hydrolysis
6,3′,4′,-trihydroxy-4-O-β-D-glucopyranosylaurone (cernuoside)	+	+	–	m p, UV, IR, ^{1}H NMR, FDMS, hydrolysis
(E)-3,4-dihydroxy cinnamic acid (caffeic acid)	+	+	+	m p, UV, IR, co-spot
6-C-β-D-glucopyranosylluteolin (iso-orientin)	–	+	–	m p, UV, ^{1}H NMR, co-spot
7-O-β-D-glucopyranosylluteolin (luteolin-7-O-glucoside)	–	–	+	m p, UV, IR, hydrolysis, co-spot

+, Present; –, Absent; m p, Melting point.

National Institute of Health, USA Bractein exhibited a certain amount of activity, but the level was not significant to warrant detailed study.

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MEETINGS/SYMPOSIA/SEMINARS

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Place: Adam's Mark Hotel
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Date: 23–26 October 1995

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Institution of Chemists (India): Associateship Examination, 1996

The Associateship Examination of the Institution of Chemists (India) will be held in November 1996. The last date for registration is 30 November 1995. For further enquiries regarding this and for Membership:

Contact: The Honorary Secretary
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Dr P. D. Gupta
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