CHAPTER II

POLYPHENOLIC COMPONENTS OF FLOWERS OF

HELICHRYSUM BRACETATUM

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POLYPHENOLIC COMPONENTS OF FLOWERS OF
HELICHRYSUM BRACETATUM

INTRODUCTION

The Asteraceae\(^1\), considered to be most advanced taxon of dicots is the largest family of vascular plants comprising about 950 genera and around 20,000 species. Members of Asteraceae are distributed over most of the earth and in all habitats. The greater proportion are herbaceous, although about 2 per cent are trees or shrubs. Economically the family is of considerable importance as its plants find use in medicinal or patented preparations; more than 215 genera are offered in the American trade as ornamentals.

Asteraceae is composed of two primary sub-divisions (i) Tubiflorae with 12 tribes including Inuleae which is characterized by all or only the central flowers tubular, the disc flowers commonly yellow and anthers tailed (ii) Liguliflorae with a single tribe Cichorieae. The Helichrysum genus under the tribe Inuleae comprises about 300 species distributed throughout South, tropical and North Africa, South Europe, Mediterranean region and in a few parts of India\(^2\).

Asteraceae, one of the largest of plant families is found to be very rich in chemical constituents\(^3\). A large
number of highly substituted flavones and flavonols have been reported\textsuperscript{4,5} from this family. Another distinctive feature is the regular occurrence of chalcones and aurone pigments in few tribes\textsuperscript{3}. The chemosystematic analysis of tribes of Asteraceae in regard to distribution of sesquiterpene lactones and flavonoids has been reviewed\textsuperscript{4}.

\textbf{Helichrysum} is characterized chiefly by the scarious glistening bracts which stand up round the flower-head (cudweed) or spread out as a white border round it (everlasting), by the arrangement of the heads in close masses and by a cottony covering on all green parts. Florets are all tubular and slender. Outer ones female (without stamens) but fertile; inner with stamens and style but infertile. Achenes are with pappus of simple hairs. \textbf{Helichrysum} can be distinguished from the closely resembling genus \textit{Anaphalis} by the leaves having 5 to 7 veins from the base, strongly impressed on the upper surface\textsuperscript{2,6}.

Many species of \textbf{Helichrysum} are reported to have good medicinal value\textsuperscript{7-17}. The leaves and flowers of \textit{H. Sanguineum} are found\textsuperscript{7} to possess anti-cancer properties; diuretic activity of several preparations from different species of \textbf{Helichrysum} has been reported\textsuperscript{8,9}. Fourteen species growing in Turkey\textsuperscript{10} exhibited anti-bacterial activity\textsuperscript{10,11,17}. Methylated flavonoids isolated from \textit{H. nitens}
are reported\textsuperscript{12,16} to possess anti-fungal properties. \textit{H. itaticum} is used in various traditional medicines to combat gallstones\textsuperscript{13}. There are reports on \textit{H. bracteatum} being active on the cardiovascular system\textsuperscript{14} and isolation of unusual biologically active chlorophenols from South African \textit{Helichrysum} species\textsuperscript{15}.

Many \textit{Helichrysum} species are found to contain terpenoids\textsuperscript{17-19}, phloroglucinols\textsuperscript{20-23}, phenylpropanoids\textsuperscript{24}, pyrone derivatives\textsuperscript{23,25} flavonoids\textsuperscript{9,18,21,26-32} and lignins\textsuperscript{34}. Among the phenolic compounds many \textit{Helichrysum} species are reported to contain cinnamic acid derivatives\textsuperscript{24}, flavones\textsuperscript{18,21,28}, chalcones and aurones\textsuperscript{26,31} and anthocyanins\textsuperscript{32}.

\textit{Helichrysum bracteatum}\textsuperscript{33} is a stout annual herb, naturalized in high altitudes; lower part of the stem is clothed with dead leaves, middle part with leaves densely woolly on both sides but veins clearly impressed and upper part is under the flower heads nearly bare of leaves. The flowers are large, everlasting and appearing golden yellow, pink and ivory white shades.

\textit{Helichrysum bracteatum} var. \textit{bracteatum} is common in Nilgiris and Kodaikanal hills of South India, growing in dense masses colouring the hill-side with its golden yellow,
pink and ivory white flowers (See Fig.1 & 2 for colour plates).

Previous report reveals that H. bracteatum contains lignins$^{34}$ and aurones$^{35,36}$. As there is no record of isolation of any other constituents and in view of the reported medicinal uses of Helichrysum species, three differently coloured (golden yellow, pink and ivory white) flowers were systematically investigated for their polyphenolics and the results are presented in this chapter.
Figure 1

HELICHRYSUM BRACTEATUM - Golden Yellow
Figure 2

HELICHRYSUM BRACTEATUM - Ivory White
Phytochemical Examination of Golden Yellow Flowers

Fresh golden yellow flowers of *Helichrysum bracteatum* var. *bracteatum* collected from the Nilgiris in South India were refluxed with 90 per cent boiling ethanol and concentrated under reduced pressure. The aqueous crude extract was factionated into benzene, ether and ethylacetate solubles. Ether and ethylacetate fractions gave positive tests for polyphenolics\(^{36-39}\) and were found to be identical in composition by PC (50% HOAc). Hence they were mixed and kept in an ice-chest for 24 h when orange-yellow solid separated. The solid on PC and TLC (cellulose) indicated the presence of four UV active compounds. Their separation into homogeneous components was achieved using preparative cellulose TLC with 50% HOAc as developing solvent. The four bands representing four compounds designated A (hRf:31), B(42), C(65) and D(70) were eluted with hot MeOH and the residue recrystallized from MeOH.

Characterization of Compound A
(5,7,3',4'-tetrahydroxyflavone: Luteolin)

Compound A, Pale Yellow needles, mp 328-330°, \(\text{C}_{15}\text{H}_{10}\text{O}_{6}\), gave yellow colour with \(\text{NH}_3\), olive green with \(\text{Fe}^{3+}\), red with \(\text{Mg-HCl}\) - all characteristic reactions of
flavonoid\textsuperscript{40-43}. It was purple under UV light changing to yellow under UV/NH\textsubscript{3}. It had Rf value (Table I) characteristic of a flavone aglycone\textsuperscript{43}. Formation of a tetraacetate (mp. 226-227\textdegree) and a tetramethyl ether (mp. 190-192\textdegree) of the compound showed the presence of four phenolic OH groups in the molecule. It had \(\lambda_{\text{max}}\) (MeOH) 242 sh, 253, 267, 291 sh and 348 nm. The hypsochromic shift of 41 nm in band I of AlCl\textsubscript{3}/HCl spectrum compared to AlCl\textsubscript{3} spectrum suggested ortho dihydroxy in ring-B. Bathochromic shift of 37 nm in AlCl\textsubscript{3}/HCl spectrum compared to MeOH spectrum revealed the presence of free 5-OH. Bathochromic shift of 53 nm in band I of NaOMe spectrum and 16 nm in band II of NaOAc spectrum indicated the presence of free 4'-OH and 7-OH respectively. The ElMS of the compound further supported the 5,7,3',4',-tetrahydroxyflavone structure. Thus compound A was characterized as 5,7,3',4'-tetrahydroxyflavone (luteolin) \((\text{Ia})\). The identity was further confirmed by direct comparison including co-PC with an authentic sample.

**Characterization of compound B**  
(6,3',4',5'-tetrahydroxy-4-O-\(\beta\)-D-glucopyranosylaurone: Bractein)

Compound B, golden yellow needles, mp. 242\textdegree\ (dec), \(C_{21}H_{20}O_{12}\) gave intense red colour with alkali, light red with Mg-HCl and positive Molisch's test. It was yellow under
UV changing to bright orange-red with UV/NH₃, suggesting it to be chalcone or aurone. It had λ_max(MeOH) 260, 325 sh and 406 nm. The dominant band I (406 nm) and relatively minor band II (260 nm) in UV-VIS spectrum was indicative of aurone. A bathochromic shift of 12 nm in NaOAc suggested free 6-OH. Also the bathochromic shift of 40 nm in band I of AlCl₃ and absence of any shift in AlCl₃/HCl spectrum compared to MeOH spectrum revealed the ortho-dihydroxyl system in B-ring with no free 4-OH. NaOAc/H₃BO₃ spectrum showed 26 nm bathochromic shift indicating the presence of ortho dihydorxy in B-ring.

The high resolution ¹H NMR spectrum (400 MHz, CDCl₃/CD₃OD, TMS, δ, ppm) exhibited signals characteristic of aurone glucoside. The doublet at 4.66 (J=8Hz) was due to anomic glucose proton and multiplet at 3.65-3.17 due to other sugar protons. The singlet at 6.31 clearly revealed the benzylic (=CH-) proton of aurones. The singlet at 6.71 for two protons corresponded to H-2' and H-6' in a 3',4',5'-trioxygented system. Doublets at 6.04 and 5.98 (J=2Hz) could be assigned to H-7 and H-5 respectively. The mass spectrum (FDMS) exhibited peaks at m/z 487 (M+Na⁺), 465 (M+H⁺) and 303 (Aglycone+H⁺).

Acid hydrolysis (2N HCl, 100°, 3 h) yielded an aglycone and sugar. The aglycone, C₁₅H₁₀O₇, mp. 350° (dec),
gave olive green colour with Fe\textsuperscript{3+}, intense red with alkali and red with Mg-HCl. It was fluorescent yellow under UV changing to orange-red under UV/NH\textsubscript{3}. It had $\lambda_{\text{max}}$ (MeOH) 262, 332 and 404 nm and exhibited characteristic shift with AlCl\textsubscript{3} (300, 350 and 504 nm) and AlCl\textsubscript{3}/HCl (230 sh, 320, 362 and 460 nm) indicating the presence of -OH at C-4, C-3', C-4' and C-5'. EIMS gave peaks at m/z 302 (C\textsubscript{15}H\textsubscript{10}O\textsubscript{7}, 100%) along with other characteristic fragment ions at 284 (M$^+\text{-H}_2\text{O}$), 274 (M$^+\text{-CO}$), 153 (A\textsubscript{1}+H), 152 (A\textsubscript{1}) 134 (A\textsubscript{1}-H\textsubscript{2}O), 125 (A\textsubscript{1}+H-CO) and 124 (A\textsubscript{1}-CO) characteristic of the aglycone as 4,6,3',4',5'-pentahydroxyaurone (bracteatin)(IIa).

The sugar was identified as D-glucose by Rf (Table-4) and confirmed by co-PC with an authentic sample. Enzyme hydrolysis\textsuperscript{42} with $\beta$-glucosidase also yielded the same products of acid hydrolysis.

The position of glycosylation was determined by a judicious interpretation of the UV-VIS spectra of the compound in MeOH and with diagnostic reagents. Bathochromic shift of 12 nm in NaOAc spectrum suggesting free 6-OH\textsuperscript{41-42}. A significant bathochromic shift of 40 nm in band I on addition of AlCl\textsubscript{3} revealed the presence of 4-OH and (or) ortho dihydroxyl in ring-B\textsuperscript{41}. With AlCl\textsubscript{3}/HCl it exhibited no shift indicating that the original shift with AlCl\textsubscript{3} alone was due to ortho dihydroxyl, suggesting glycosylation at 4-OH (compare aglycone).
Thus, compound B was characterized as 6,3',4',5'-tetrahydroxy-4-O-β-D-glucopyranosylaurone (bractein) (IIb) earlier isolated from *H. bracteatum*.

**Characterization of Compound C**
(6,3',4'-trihydroxy-4-O-β-D-glucopyranosylaurone: Cernusoside)

Compound C, yellow needles, mp. 260°C (dec), *C_{21}H_{20}O_{11}*, gave red colour with alkali and Mg-HCl and positive Molisch's test. It was bright yellow under UV changing to orange-red under UV/NH_{3} and had Rf similar to compound B indicating it to be an aurone. Its \( \lambda_{\text{max}}(\text{MeOH}) \) 262, 338 and 404 nm with high intensity band I and Rf (Table 3) were typical of aurone glycoside.

A bathochromic shift of 10 nm in band II of NaOAc suggested free 6-OH. Further, the bathochromic shift of 32 nm in band I of AlCl\(_{3}\) and absence of any shift in AlCl\(_{3}/\)HCl compared to MeOH indicated the presence of ortho dihydroxyl in ring-B and absence of free 4-OH.

The high resolution \(^1H\) NMR (400 MHz, CDCl\(_3/\)CD\(_3\)OD, TMS, \( \delta \), ppm) exhibited signals at 4.68 corresponding to anomeric sugar proton and 3.68-3.19 due to other sugar protons of β-D-glucoside. The singlet at 6.42 revealed benzylic proton of aurone (-CH=) and doublet (J=2Hz) at 7.22 was assigned to H-2'. Further signals at 6.98 (1H, dd,
J=8 and 2Hz) and 6.60 (1H, d, J=8Hz) were due to H-6' and H-5' respectively. The doublets (J=2Hz) at 6.09 and 6.02 were due to H-7 and H-5 respectively. FDMS showed peaks at 470 (M+Na+), 449 (M+H+) and 287 (aglycone+H+).

Acid hydrolysis (2N HCl, 100°, 3 h) gave aglycone and sugar. Aglycone, C_{15}H_{10}O_{6}, mp. 274-276° gave red colour with alkali, olive green with Fe^{3+}, light red with Mg-HCl. It was fluorescent yellow under UV changing to orange-red with UV/NH_{3}. It had λ_max (MeOH) 260, 332, and 399 nm. The characteristic shifts with diagnostic shift reagents (as detailed for glycoside) suggested a free 4-OH and 3',4'-ortho dihydroxyl, and free 6-OH. The EIMS exhibited molecular ion peak at m/z 286 (M^+, C_{15}H_{10}O_{6}, 100%), 268 (M^+-H_2O) 258 (M^+-CO), 153 (A_1+H), 152 (A_1), 125 (A_1+H-CO) and 124 (A_1-CO) indicating the aglycone to be 5'-deoxy aglycone of B. Thus, aglycone of C was constituted as 4,6,3',4'-tetra-hydroxyaurone (aureusidin) (IIc).

The sugar was identified as D-glucose by Rf (Table-4) and confirmed by co-PC with an authentic sample. Enzyme, β-glucosidase hydrolysed compound C to aureusidin, showing C is a β-D-glucoside of aureusidin.

The position of glycosylation was determined by interpretation of the UV-VIS spectra of the compound in MeOH and with shift reagents. Bathochromic shift of 10 nm in
band II of NaOAc spectrum revealed free 6-OH. With AlCl$_3$ it gave bathochromic shift of 32 nm in band I showing the presence of 4-OH and/or 3',4'-ortho dihydroxyl. AlCl$_3$/HCl spectrum did not give any shift indicating that the original shift with AlCl$_3$ alone was due to ortho dihydroxyl and not due to 4-OH, suggesting glycosylation at 4-OH (compare aglycone).

Thus, compound C was characterized as 6,3',4'-tri-hydroxy-4-O-β-D-glucopyranosylaurone (cernuoside) (IIId), earlier isolated from Oxalis cernua.$^{44}$

**Characterization of Compound D**

((E)-3,4-dihydroxycinnamic acid: (E)-caffeic acid)

Compound D, colourless needles, mp. 210-211°, C$_9$H$_8$O$_4$, gave brisk effervescence with saturated solution of HCO$_3$⁻, decolourised Br$_2$/H$_2$O and brownish blue colour with Fe$_3$⁺. It was blue under UV changing to bright blue under UV/NH$_3$ . It had λ max(MeOH) 235 sh 288 and 315 nm. Acetylation of this compound yielded a diacetate, mp. 201-203°. $^1$H NMR spectrum gave signals for 1,3,4-trisubstituted benzene derivative. ($\delta$, 7.16, d, $J$=2.5 Hz, 1H, H-2; 7.03, dd, $J$=8.3 and 2.3 Hz, 1H, H-6; 6.86, d, $J$=8.3 Hz, 1H, H-5). The trans-stereochemistry was deduced from the peaks at $\delta$ 7.53 and 6.26 ( d, $J$=15.9 Hz).
The mass spectrum having molecular ion peak at m/z 180 along with other characteristic peaks at 163, 136, 134, 69, 57 and 55 indicated the presence of benzene ring with two-OH groups and a side chain -CH=CH-COOH. Thus compound E was characterized as (E)-3,4-dihydroxy cinnamic acid ((E)-caffeic acid) (III) whose identity was confirmed by co-PC and superimposable IR spectra with an authentic sample 45.
Phytochemical Examination of Pink Flowers

Fresh pink flowers of *H. bracteatum*, collected from Kotagiri of Nilgiri hills (Tamil Nadu) were extracted as in the case of golden yellow flowers *H. bracteatum*. The crude extract was chromatographed on column (SiO₂) and eluted with CHCl₃ and CHCl₃ containing increasing percentage of MeOH. CHCl₃: MeOH (3:1) fraction gave a mixture of three compounds, which was separated into individual compounds by preparative PC (Whatman No.3, 50% HOAc, descending, 28°, 9 h). One purple band (hRf: 30), two bright yellow bands (43 and 64) and a blue band (72) were detected under UV light. The CHCl₃: MeOH (1:3) fraction yielded a pale yellow additional compound which was crystallized from MeOH. The four compounds from CHCl₃: MeOH (3:1) eluate were identified as luteolin, bractein, cernuoside and caffeic acid present in the golden yellow flowers. The characterization of the fifth compound (E) is given below.

Characterization of Compound E
(6-C-β-D-glucopyranosylluteolin: Iso-orientin)

Compound E, mp. 236-237°, C₂₁H₂₀O₁₁, [α]D²⁸ +30.8° (c.0.5, pyridine), gave yellow colour with alkali, pink with Mg-HCl, olive green with Fe³⁺ and positive Molisch's test. It was purple under UV and yellow under UV/NH₃. Its mobility on PC showed it to be a flavone glycoside. It did
not undergo hydrolysis on heating with 2N HCl (100°, 4h) suggesting it to be a C-glycosylflavone\textsuperscript{41-43}. The $\lambda_{\text{max}}$(MeOH) 255, 269, 348 and diagnostic shifts with various shift reagents (see experimental), were very similar to those of luteolin and orientin. When subjected to hydrolytic fission with HI, luteolin was obtained as the aglycone. The $^1$H NMR spectrum of compound E in DMSO-$d_6$ had signals for aromatic protons at 3,8,2',5',6' and aliphatic protons of glucose; the anomeric proton being found at $\delta$ 4.57 as a doublet ($J=9\text{Hz}$) characteristic of $\beta$-configuration in C-$\beta$-D-gluco-pyranosylflavones\textsuperscript{41-43}. Acetylation with Ac$_2$O and pyridine at room temperature gave a mixture of acetates whereas sodium acetate-acetic anhydride method yielded a peracetate, mp. 140-142° indicating probable 6-C-glycosylation. The mass spectrum of the permethyl ether of E exhibited the parent ion at m/z 560 (26%) and the base peak at m/z 385 (M-175) confirming it to be 6-C-glucosyl-luteolin. Based on the above data compound E was characterized as 6-C-$\beta$-D-glucopyranosylluteolin (iso-orientin) (Ib) and its identity was fully established by direct comparison, including co-PC and super-imposable IR spectra with an authentic sample from \textit{Parkinsonia aculeata}\textsuperscript{46}. 
Phytochemical Examination of Ivory White

Flowers of *H. bracteatum*

Fresh ivory white flowers of *H. bracteatum* (Fig. 2) collected from Botanical Garden, Ooty (South India) were extracted and fractionated as in the case of golden yellow and pink flowers of *H. bracteatum*. Ether fraction was found to contain two compounds, separated by PPC (Whatman No. 3, 15% HOAc, decending, 28°, 8h). The purple band under UV light (hRf: 6) and blue band (50) were found to be luteolin and caffeic acid as in golden yellow and pink flowers. EtOAc and MeCOEt extracts showing identical spots on PC were mixed and separated by column chromatography (SiO₂; CHCl₃ and CHCl₃ containing increased concentration of MeOH). CHCl₃:MeOH (6:4) fraction yielded a pale yellow compound (F) whose characterization is given below. CHCl₃:MeOH (1:9) fraction yielded a yellow compound identified as iso-orientin.

**Characterization of Compound F**
(7-O-β-D-glucopyranosylluteolin)

Compound F, pale yellow needles, mp. 251-253°, C₂₁H₂₀O₁₁, gave yellow colour with alkali, olive green with Fe³⁺ and red with Mg/HCl. It answered Molisch's test. It was purple under UV changing to yellow with UV/NH₃. It had λmax(MeOH) 255, 267 sh and 348 nm typical of flavones.
Acid hydrolysis (2N HCl, 100°, 2h) yielded aglycone identified as luteolin and sugar as D-glucose as explained earlier. Enzyme hydrolysis (β-glucosidase) also yielded same products showing the compound as a β-D-glucoside of luteolin.

The site of glycosylation was established by interpretation of UV spectrum in MeOH and in presence of diagnostic reagents. The UV spectrum in MeOH of glycoside (255, 267 sh, 348 nm) and aglycone (253, 267, 291 sh, 348 nm) were almost same suggesting 7-OH was involved in glycosylation. Band I of AlCl₃ spectrum, (432 nm) and AlCl₃/HCl spectrum (387 nm) for both the glycoside and aglycone was almost same indicating unalteration of free 3', 4' and 5-OH, leaving 7-OH for glycosylation. The NaOAc spectrum did not show significant shift of band II (Δλ max, 4nm) in agreement with 7-O-glycosylation. Thus, compound F, was characterized as 5,3',4'-trihydroxy-7-0-β-D-glucopyranosylflavone (luteolin-7-O-β-D-glucoside) (Ic) and the identity was confirmed by co-PC with an authentic sample.
a) \( R_1 = R_2 = H \): Luteolin
b) \( R_1 = H; R_2 = \beta-D\text{-glucopyranosyl} \): Iso-orientin
c) \( R_1 = \beta-D\text{-glucopyranosyl}; R_2 = H \): Luteolin-7-O-\( \beta-D\text{-glucoside} \)

a) \( R_1 = H; R_2 = \text{OH} \): Bracteatin
b) \( R_1 = \beta-D\text{-glucopyranosyl}; R_2 = \text{OH} \): Bractein
c) \( R_1 = R_2 = H \): Aureusidin
d) \( R_1 = \beta-D\text{-glucopyranosyl}; R_2 = H \): Cernuoside
(E) - caffeic acid

β-D-glucopyranoside
RESULTS AND DISCUSSION

Systematic examination of golden yellow, pink and ivory white flowers of *H. bracteatum* for their polyphenolics yielded positive results. The golden yellow flowers has been found to contain a flavone, two aurone glycosides and a phenylpropanoic acid. These were characterized as 5,7,3',4'-tetrahydroxyflavone (luteolin), 6,3',4',5'-tetrahydroxy-4-0-β-D-glucopyranosylaurone (bractein), 6,3',4'-trihydroxy-4-0-β-D-glucopyranosylaurone (cernuoside) and (E)-3,4-dihydroxycinnamic acid (caffeic acid). Examination of pink flowers yielded an additional flavone glycoside characterized as 5,7,3',4'-tetrahydroxy-6-C-β-D-glucopyranosylflavone (iso-orientin). The ivory white flowers were devoid of aurones but contained luteolin, two of its glycosides and caffeic acid. The glycosides were identified as 5,3',4'-trihydroxy-7-O-β-D-glucopyranosylflavone (luteolin-7-O-glucoside) and iso-orientin. The three varieties of *H. bracteatum* were found to be different in their flavonoid composition.

Except bractein all other compounds were isolated for the first time from *H. bracteatum*. Present isolation of bractein from the Indian variety is in agreement with its earlier occurrence in the European variety. The structures of bractein and cernuoside were established by studying the
colour reactions, observation of UV fluorescence, careful analysis of spectroscopic data including high resolution $^1$H NMR and mass spectra and standard chemical methods. In other cases identification of the compounds based on spectral and chemical analysis was confirmed by direct comparison with authentic samples.

The two aurone glycosides, B and C on acid and enzyme ($\beta$-glucosidase) hydrolysis yielded same sugar (D-glucose) but different aglycones (bracteatin from B and aureusidin from C) showing them as $\beta$-D—glucoside of bracteatin and $\beta$-D-glucoside of aureusidin respectively. The position of glycosylation in both the compounds was determined by a judicious interpretation of the UV-VIS spectra of the aglycones and glycosides in MeOH and with diagnostic shift reagents.

Bathochromic shift in NaOAc spectrum suggested free 6-OH. Further, significant bathochromic shift in $\text{AlCl}_3$ spectrum revealed the presence of 4-OH and / or orthodihydroxyl in ring B$^{41}$. The $\text{AlCl}_3$/HCl spectrum was unaltered from MeOH spectrum revealing the absence of free 4-OH and thus pointing to its involvement in glycosylation.

The high resolution $^1$H NMR spectrum of compound B exhibited signal at $\delta$ 4.68 (1H, d, $J$=8 Hz) corresponding to anomeric proton of $\beta$-D-glucoside$^{41}$. The singlet at $\delta$ 6.31
revealed the benzylic proton of aurone and singlet at δ 6.71 integrating for two protons are of 2' and 6'-proton of B-ring. The doublets at δ 6.06 and 5.98 with J=2Hz were due to H-7 and H-5 respectively. Further evidence was obtained from FDMS data, exhibiting prominent ions at m/z 487 (M+Na⁺), 465 (M+H⁺) and 303 (aglycone + H⁺). Thus compound B was constituted as 6,3',4',5'-tri hydroxy-4-0-β-D-glucopyranosylaurone (bractein) 35.

1H NMR spectrum of compound C exhibited signals at δ 7.22 (d, J=8 Hz, 1H) 6.98 (dd, J=8 and 2 Hz, 1H), 6.60 (d, J=8 Hz, 1H) corresponding to protons at 2',6' and 5'. The benzylic proton appeared at δ 6.42 as singlet, H-7 at δ 6.09 (d, J=2 Hz, 1H) and H-5 at δ 6.02 (d, J=2 Hz, 1H). The doublet at δ 4.68 (J=9 Hz) was due to the anomeric proton of β-D-glucose. FDMS spectrum showing peaks at m/z 471 (M+Na⁺), 449 (M+H⁺) and 287 (aglycone + H⁺) gave additional evidence for the structure of C as 5'-deoxybractein. Thus compound C was characterized as 6,3',4'-trihydroxy-4-0-β-D-glucopyranosylaurone (cernuoside) earlier isolated from Oxalis cernua 44.

Distribution of flavonoids in Asteraceae 3-5 reveals the predominance of apigenin and luteolin along with their 7-glycosides. The present finding of luteolin and its 7-glucoside is in further support.
The co-occurrence of phenylpropanoid, aurones and flavones in *H. bracteatum* is in agreement with the biogenesis of these types of compounds involving common precursors like mevalonic acid, shikimic acid and phenylalanine. Though aurones and chalcones listed under minor flavonoids are known to be chemotaxonomically important compounds and of general value in regard to the family Asteraceae, their applicability at sub family level is limited as they have been found to occur in the sub tribe Corepsidinae of the tribe Helenieae and also in Verbesiniae, Cynareae and Inuleae.

The present study has revealed that three morphologically similar species of *H. bracteatum*, differing only in colour of the flowers, biosynthesize three different flavonoid compounds—aurone in golden yellow, aurone and flavone in pink and flavone in ivory white varieties.

Some of the biological activity reported for *H. bracteatum* may be due to flavonoids which are known for their medicinal properties (chapter-I).
**EXPERIMENTAL***

_H. bracteatum_ - Golden Yellow Flowers

Fresh flowers of _H. bracteatum_ (600 g) collected from Dodabetta peak of Nilgiri Hills in South India, were exhaustively extracted with hot 80 per cent EtOH (3 x 4 l) under reflux and the combined extracts concentrated in vacuo. The aqueous alcoholic concentrate (2-3 g) was fractionated using C\textsubscript{6}H\textsubscript{6} (3 x 0.5 l), Et\textsubscript{2}O (6 x 0.5 l) and EtOAc (4 x 0.5 l). C\textsubscript{6}H\textsubscript{6} fraction did not yield any crystalline compound. Et\textsubscript{2}O concentrate when kept in an ice chest yielded an intense yellow solid (500 mg). On PC (50% Melting points reported are uncorrected and determined in open capillaries. Rf values are determined on Whatman No.1 paper (ascending) in different developing solvents as mean of at least three runs and indicated as hRf (Rf x 100). In the majority of cases, compounds are detected under UV light alone or after exposure to NH\textsubscript{3} vapours and also recorded as UV fluorescence. UV-VISIBLE absorption spectra are measured in spectroscopic MeOH and in presence of diagnostic shift reagents in the range of 220-470 nm. No molar extinction coefficient (\epsilon) is indicated for uniformity although determined in a few cases. IR spectra have been recorded using KBr pellets unless otherwise indicated, in the range of 4000 - 650 cm\textsuperscript{-1}. \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectra are recorded in CDCl\textsubscript{3} or CDCl\textsubscript{3}/CD\textsubscript{3}OD or DMSO-d\textsubscript{6} or DMSO-d\textsubscript{6} / D\textsubscript{2}O and chemical shifts are given in \delta scale. Mass spectral data have been obtained by electron impact (EI) or field desorption (PD) or fast atom bombardment (FAB) methods and presented as normalized spectrum with m/z versus intensity. All the crystalline compounds gave satisfactory elemental analysis. The specific enzymes used for hydrolysis have been obtained from commercial sources like Sigma, Park-Davis, etc.
HOAc), this showed four ployphenolic compounds. These were separated by preparative TLC using microcrystalline cellulose powder.

The plates were prepared by mixing 20 g of cellulose powder (microcrystalline) in 140 ml of water and homogenization in a mechanical mixer for 3-5 min. Coating was done using standard TLC applicator on glass plate (20 x 20 cm) with film thickness adjusted to 0.5 mm. The plate was gently rocked until the material was evenly distributed over the surface. After air-drying (28-30°) for about 5 h the plate was ready for use.

The mixture was dissolved in minimum quantity of MeOH and applied on chromoplates by an improvised applicator along a line 2 cm from the starting side of the plate. After allowing the band to air-dry, the plates were developed using 50 per cent aqueous HOAc as solvent. The four zones with hRf:31, 42, 65 and 70 designated as compounds A, B, C and D were demarcated under UV light and separated. The compounds were eluted with MeOH and the MeOH eluate freed from cellulose particles by centrifugation and concentrated.
Compound A  
(Luteolin)

Pale yellow needles (MeOH), mp. 329-331° (110 mg)  
(Found: C, 62.7; H, 3.6; C_{15}H_{10}O_{6} requires C, 62.93; H, 3.49%), gave yellow colour with NH₃, Na₂CO₃ and NaOH, olive green with Fe⁢³⁺ and red with Mg-HCl. It was purple under UV light and yellow with UV/NH₃.

\textbf{UV} (λ_{max.}, nm)

\begin{align*}
\text{MeOH} & : 242 \text{ sh}, 253, 267, 291 \text{ sh}, 348 \\
+ \text{ NaOAc} & : 269, 326 \text{ sh}, 384 \\
+ \text{ NaOAc} + \text{ H₃BO₃} & : 259, 301 \text{ sh}, 370, 432 \text{ sh} \\
+ \text{ NaOMe} & : 266 \text{ sh}, 329 \text{ sh}, 401 \\
+ \text{ AlCl₃} & : 274, 300 \text{ sh}, 328, 426 \\
+ \text{ AlCl₃} + \text{ HCl} & : 266 \text{ sh}, 275, 294 \text{ sh}, 355, 385.
\end{align*}

\textbf{IR} (ν_{max.}, cm⁻¹, KBr)

3400 br, 2910, 1725, 1650, 1610, 1500, 1450, 1360, 1250, 1200, 1190, 950, 900, 850, 810 and 750.

Rf: Table 3

\textbf{Acetylation}  
(Luteolin tetraacetate)

Compound A (25 mg) dissolved in C₅H₅N (0.5 ml) was treated with Ac₂O (0.5 ml) and left at room temperature for 24 h. It was poured into crushed ice in a beaker with
continuous stirring. After keeping in ice for 3 h, the solid obtained was filtered, washed with distilled water and dried. Colourless needles (MeOH), mp. 226-227° (30 mg).

**Methylation**
*(Luteolin tetramethyl ether)*

Compound A (25 mg) in Me₂SO₄ (1.0 ml), anhydrous K₂CO₃ (1g) and Me₂CO (20 ml) was refluxed at 70° for 36 h. The reaction product was cooled, filtered at the pump and the residue well washed with warm Me₂CO. The Me₂CO filtrate was concentrated and mixed with H₂O. The light yellow solid obtained was filtered and recrystallized from MeOH to yield colourless needels, mp. 190-192° (31 mg).

**Trimethylsilylation**

Compound A (25 mg) was treated with 5 ml C₅H₅N and 5 ml hexamethyldisilazane, to which 5 ml of (CH₃)₃ SiCl was added and the flask kept stoppered for 1 h. The contents were dried in rotary evaporator and the residue was taken in CC₁₄.

**¹H NMR of TMS ether of Compound A** *(60 MHz, CC₁₄, TMS as internal standard, δ, ppm)*

7.35 (unresolved s, 2H, H-2' and 6'), 6.80 (d, J=8.5Hz, 1H, H-5'), 6.45 (d, J=2.5Hz, 1H, H-8), 6.30 (s, 1H, H-3), 6.15 (d, J=2.5Hz, H-6), 0.20 (m, 36 H, 4 x (CH₃)₃Si)


**Compound B**
(Bractein)

Golden yellow needles (MeOH), mp. 242° (dec) (60 mg), gave intense red colour with NaOH, red with Mg-HCl and positive Molisch's test. It was fluorescent yellow under UV light changing to orange red on exposure to NH₃.

**UV (λmax., nm)**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>260, 325 sh, 406</td>
</tr>
<tr>
<td>+ NaOAc</td>
<td>272, 325 sh, 420</td>
</tr>
<tr>
<td>+ NaOAc + H₃BO₃</td>
<td>270, 320 sh, 432</td>
</tr>
<tr>
<td>+ NaOMe</td>
<td>294, 445 (dec)</td>
</tr>
<tr>
<td>+ AlCl₃</td>
<td>255, 287, 338 sh, 446</td>
</tr>
<tr>
<td>+ AlCl₃ + HCl</td>
<td>254, 273, 338 sh, 389, 409.</td>
</tr>
</tbody>
</table>

**¹H NMR (400 MHz, CDCl₃/CD₃OD, TMS as int.std, δ, ppm)**

(See Fig.3).

6.71 (s, 2H, H-2' and 6'), 6.31 (s, 1H, =CH-), 6.04 (d, J=2Hz, 1H, H-7), 5.98 (d, J=2 Hz, 1H, H-5), 4.66 (d, J=8Hz, 1H, anomeric glucose proton), 3.65-3.17 (m, 6H, other glucose protons).

**FDMS (m/z values)**

487 (M+Na⁺), 465 (M+H⁺) and 303 (aglycone +H⁺)

Rf: Table 3.
Acid Hydrolysis of Compound B
(Bracteatin and D-glucose)

20 mg of the compound B was dissolved in hot MeOH and equal volume of 4N HCl was added. The reaction mixture was refluxed at 100° for 3h. The excess MeOH was distilled off in vacuo and the resulting aqueous solution diluted with H2O and left in the ice chest for 5 h. The solid separated out was filtered, washed with cold water and dried. The aqueous filtrate was shaken with Et2O and the residue from ether extract was combined with solid on the filter and total aglycone weighed (11 mg).

The aqueous part was neutralised with PbCO3, filtered through Whatman No.42 filter paper, passed through column of Amberlite (120 (H+) resin to remove lead ions and then concentrated. It was subjected to PC and co-PC with authentic sample of D-glucose; both showed identical Rf (Table 4)

Enzyme hydrolysis of Compound B
(Bracteatin and D-glucose)

An aqueous solution of compound B (5 mg) was mixed with β-glucosidase (1 mg) in 2 ml of 0.1 M HOAc:NaOAc buffer (1 ml) (pH: 5.0) and kept at 37° for 24 h. The aglycone and sugar were same as those from acid hydrolysis.
**A glycone of Compound B**  
(Bracteatin)

Bright yellow needles (MeOH), mp. 350° (dec), gave red colour with NaOH, olive green with Fe^3+, dull red in Mg-HCl. It was fluorescent yellow under UV changing to orange-red under UV/NH₃.

**UV (λ max., nm)**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>λ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>262, 332, 404</td>
</tr>
<tr>
<td>+ NaOAc</td>
<td>276, 340 sh, 440</td>
</tr>
<tr>
<td>+ NaOAc + H₃BO₃</td>
<td>276, 322, 403</td>
</tr>
<tr>
<td>+ NaOMe</td>
<td>Unstable</td>
</tr>
<tr>
<td>+ AlCl₃</td>
<td>300, 350, 504</td>
</tr>
<tr>
<td>+ AlCl₃ + HCl</td>
<td>230 sh, 320, 362, 460</td>
</tr>
</tbody>
</table>

**EI MS (70 eV, m/z values) (See Fig.4)**

302 (M⁺, C₁₅H₁₀O₇, 100 %), 284 (M⁺-H₂O), 274 (M⁺-CO), 153 (A₁+H), 152 (A₁), 134 (A₁-H₂O), 125 (A₁+H-CO) and 124 (A₁-CO).

Rf: Table 3

**Compound C**  
(Cernuoside)

Yellow needles (MeOH), mp. 260° (dec) (40 mg), gave red colour with NaOH, light red with Mg-HCl and posi-
tive Molisch's test. It was fluorescent yellow under UV light changing to orange-red with UV/NH₃.

**UV (λ_max., nm)**

<table>
<thead>
<tr>
<th>Solution</th>
<th>λ_max (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>262, 338, 404</td>
</tr>
<tr>
<td>+ NaOAc</td>
<td>272, 342 sh, 438</td>
</tr>
<tr>
<td>+ NaOAc + H₃BO₃</td>
<td>268, 337 sh, 430</td>
</tr>
<tr>
<td>+ NaOMe</td>
<td>298, 445 (dec)</td>
</tr>
<tr>
<td>+ AlCl₃</td>
<td>274, 321, 436</td>
</tr>
<tr>
<td>+ AlCl₃ + HCl</td>
<td>266 sh, 275, 294 sh, 355, 406</td>
</tr>
</tbody>
</table>

**¹H NMR (400 MHz, CDCl₃/CD₃OD, TMS as int.std, δ, ppm)**

(See Fig. 5)

7.22 (d, J=2Hz, 1H, H-2'), 6.98 (dd, J=8 and 2 Hz, 1H, H-6'), 6.60 (d, J=8Hz, 1H, H-5'), 6.42 (s, 1H, -CH-), 6.09 (s, 1H, H-7), 6.02 (s, 1H, H-5), 4.68 (d, J=8Hz, 1H, anomeric glucose proton), 3.68-3.19 (m, 6H, other glucose protons).

**FDMS (m/z values)**

470 (M+Na⁺), 449 (M+H⁺) and 287 (aglycone +H⁺).

Rf: Table 3
Acid hydrolysis of Compound C
(Aureusidin and D-glucose)

Compound C (10 mg) on acid hydrolysis (2N HCl, 100°, 2 h) and working up as under Compound B yielded an aglycone and glucose in 1:1 ratio.

Enzyme hydrolysis

Adopting procedure followed for compound B, compound C was hydrolysed to the aglycone and D-glucose by enzyme β-glucosidase.

Aglycone of Compound C
(4,6,3',4'-tetrahydroxyaurone: Aureusidin)

Yellow needles (MeOH), mp. 274-276°, gave red colour with NaOH, olive green with Fe³⁺, light red with Mg-HCl. It was fluorescent yellow under UV light changing to orange-red under UV/NH₃.

UV (λ max., nm)

MeOH : 260, 332, 399
+ NaOAc : 275, 335 sh, 435
+ NaOAc + H₃BO₃ : 275, 332 sh, 432
+ NaOMe : Unstable
+ AlCl₃ : 295, 343, 490
+ AlCl₃ + HCl : 233 sh, 315, 448
**EIMS** (70 eV, m/z values) (See Fig.6)

286 ($M^+$, $C_{15}H_{10}O_6$, 100 %), 268 ($M^+-H_2O$), 258 ($M^+-CO$), 153 ($A_1+H$), 152 ($A_1$), 134 ($A_1-H_2O$), 125 ($A_1+H-CO$) and 124 ($A_1-CO$).

Rf: Table 3.

**Compound D**

((E)-Caffeic acid)

Crystallized from MeOH, as pale yellow needles, mp. 210-212°, gave brisk effervescence with saturated NaHCO$_3$ solution, light blue with Fe$^{3+}$ and decolourised Br$_2$ water. It was blue under UV and deep blue under UV/NH$_3$.

**UV** ( $\lambda$max., nm)

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>: 235 sh, 288, 315</td>
<td></td>
</tr>
<tr>
<td>+ AlCl$_3$</td>
<td>: 234 sh, 265, 320, 360</td>
<td></td>
</tr>
<tr>
<td>+ AlCl$_3$ + HCl</td>
<td>: 234 sh, 300 sh, 320</td>
<td></td>
</tr>
<tr>
<td>NaOH</td>
<td>: 306 sh, 334.</td>
<td></td>
</tr>
</tbody>
</table>

**IR** ( $\nu$max., cm$^{-1}$, KBr)

3440, 1640, 1505, 1212, 1172, 1118, 972, 889, 849 and 812.
\(^1\)H NMR (350 MHz, DMSO-\(d_6\), TMS as int. std, \(\delta\), ppm)

7.53 (d, \(J=15.9\) Hz, 1H, H-0), 7.16 (d, \(J=2.3\) Hz, 1H, H-2'), 7.03 (dd, \(J=8.3\) and 2.3 Hz, 1H, H-6'), 6.86 (d, \(J=8.3\) Hz, 1H, H-5') and 6.26 (d, \(J=15.9\) Hz, 1H, H-B)

MS (70 eV, m/z values)

180 (\(M^+\), 100 %), 163 (\(M^+-17\)), 136 (\(M^+-44\)), 134, 69, 57, 55.

Rf: Table 3

Acetylation
(Caffeic acid diacetate)

Compound D (25 mg) in C\(_5\)H\(_5\)N (0.5 ml) and Ac\(_2\)O (0.5 ml) left at room temperature for 24 h yielded a diacetate, colourless needles (EtOAc-petrol) mp. 201-203\(^\circ\).
**H. bracteatum** - Pink Flowers

Fresh pink flowers (300 g) collected from Pandian Raj Memorial Park in Kotagiri of Nilgiri hills, South India, were extracted with 90 per cent boiling EtOH and concentrated in vacuo. The aqueous alcoholic concentrate was kept in an ice chest to yield dark yellow solid (3.5 g). This was found to be mixture of five compounds by PC (50% HOAc). These were found to be polyphenolics and were separated by column chromatography on SiO₂. The column was developed with CHCl₃ and elution was carried out using CHCl₃ as well as mixtures of CHCl₃ with increasing proportions of MeOH as given in Table-1.

Fractions (4-20) were mixed and concentrated. This gave bright yellow solid (350 mg) which was found to be mixture of four compounds on PC (50% HOAc) separated into homogeneous compound A (luteolin), B (bractein), C (cernuose) and D (caffeic acid) present in the golden yellow flowers. Fractions (21-35) when mixed and concentrated yielded pale yellow crystals. This was found to be homogeneous, exhibited all characteristic reactions of flavonoid glycoside and designated as compound E.

**Compound E**
(Iso-orientin)

Pale yellow needles (MeOH), mp 235–236°C, [α]₂₈+30.8 (c 0.5, pyridine), (Found: C, 55.9; H, 4.8; C₂₁H₂₀O₁₁
requires C, 56.3; H, 4.5%), gave yellow colour with NH₃, Na₂CO₃ and NaOH, pink with Mg-HCl, olive green with Fe³⁺ and positive Molisch's tests. It was purple under UV and yellow under UV/NH₃.

**UV (λmax., nm)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>λmax., nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>255, 269, 348</td>
</tr>
<tr>
<td>+NaOAc</td>
<td>271, 276 sh, 330 sh, 377</td>
</tr>
<tr>
<td>+NaOAc + H₃BO₃</td>
<td>261, 372</td>
</tr>
<tr>
<td>+NaOMe</td>
<td>268, 274 sh, 346 sh, 403</td>
</tr>
<tr>
<td>+AlCl₃</td>
<td>275, 300 sh, 340 sh, 425</td>
</tr>
<tr>
<td>+AlCl₃ + HCl</td>
<td>263 sh, 277, 293 sh, 359, 385</td>
</tr>
</tbody>
</table>

**IR (νmax., cm⁻¹, KBr)**

3340 br, 1610, 1575, 1475, 1450, 1360, 1280, 1220, 1165, 1120, 1080, 1030, 980, 955, 835, 810, 755, 725 and 685.

***H NMR*** (270 MHz, DMSO-d₆, TMS as int. std, δ ppm) (Fig. 7)

13.57 (s, 1H, 5-OH), 7.43 (d, J=2 Hz, 1H, H-2'), 7.40 (dd, J=2, 8 Hz, H-6'), 6.90 (d, J=8Hz, H-5'), 6.88 (s, 1H, H-8), 6.49 (s, 1H, H-3), 4.57 (d, J=9Hz, 1H, anomeric proton of glucose), 4.0-3.2 (m, 6H, glucose protons).

Rf Table 3
Permethylation of Compound E

Compound E (25 mg) in MeI (1ml) and Ag₂O in DMF (1.5 ml) kept at room temperature for 48 h, the mixture filtered and the residue washed with a little DMF. The filtrate evaporated to dryness and the residue, purified by TLC (SiO₂), subjected to mass spectrometry.

MS (70eV, m/z values)

560 (M⁺), 545 (M⁺-15), 529 (M⁺-31), 513 (M⁺-47), 454 (M⁺-103), 427 (M⁺-133), 385 (M⁺-175 100%), 371 (M⁺-189), 355 (M⁺-205) and 341 (M⁺-219).

Acetylation of Compound E

Octaacetate (acetic anhydride and fused NaOAc, 140°, 2 hr) colourless needles, mp. 140-141°

Hydrolytic fission of Compound E
(Luteolin)

HI (2 ml) was added dropwise slowly with stirring and cooling, to a solution of compound A (20 mg) in Ac₂O (2 ml). After refluxing at 170-189° for 2 h, the reaction product was cooled and poured into a saturated solution of NaHSO₃ and extracted with ether. The residue from the dried ether on recrystallization gave yellow needles (MeOH)
mp. 328-330°, purple under UV changing to yellow under UV/NH₃. M.p., colour reactions and Rf indistinguishable from Compound A (luteolin) isolated from golden yellow and pink flowers of H. bracteatum.

**Identification of Sugar of Compound E (D-glucose)**

About 20 mg of compound E and 1g of FeCl₃ in 3 ml of water were heated under reflux at 115° for 15 min. and then at 125° for 6 h, diluted with H₂O, filtered and the pale yellow filtrate passed through a column of IRC-120 (H⁺) (10 g) followed by IRA, 400 (OH) (10 g) to remove Fe³⁺ and Cl⁻ ions. The neutral solution was concentrated and subjected to PC with authentic sugar. D-glucose and D-arabinose were identified (D-arabinose is an artefact formed from D-glucose).

Rf: Table 4


**H. bracteatum—Ivory White Flowers**

Fresh ivory white flowers of *H. bracteatum* (400 g) collected from Govt. Botanical Gardens, Ooty, South India were extracted with 85% boiling EtOH (3x3 l). The aqueous alcoholic concentrate was partitioned with C₆H₆ (3 x 1 l), Et₂O (4 x 0.5 l), EtOAc (3 x 1 l) and MeCOEt (3 x 1 l) in succession and each extract was concentrated. C₆H₆ soluble fraction did not yield any crystalline compound.

**Examination of Et₂O fraction**

Et₂O fraction showed two spots under UV on PC (15% HOAc) and was separated into individual compounds by preparative PC (Whatman No.3, descending, 15% HOAc, 28⁰, 6h). The purple band under UV (hRf:6) designated compound A (luteolin) and blue band (hRf:50) compound D (caffeic acid), were present in the golden yellow and pink flowers of *H. bracteatum*.

**Examination of EtOAc and MeCOEt extract**

The EtOAc and MeCOEt fractions were found to be identical, containing two compounds (PC). They were therefore mixed and the residue from the concentrate dissolved in a minimum quantity of MeOH. This was subjected to column chromatography (SiO₂) as detailed in Table 2.
Eluates 4-10 yielded a pale yellow compound (P). Further eluates (11-15) gave another yellow compound identified as iso-orientin, earlier identified in pink flowers of H.bracteatum.

**Compound F**
(Luteolin-7-O-β-D-glucoside)

Pale yellow needles (MeOH) mp.249-251° gave yellow colour with alkali, olive green with Fe³⁺ and pink with Mg-HCl. It answered Molisch's test and was purple under UV changing to yellow under UV/NH₃.

**UV (λ max., nm)**

<table>
<thead>
<tr>
<th></th>
<th>MeOH</th>
<th>+NaOAc</th>
<th>+NaOAc + H₃BO₃</th>
<th>+NaOMe</th>
<th>+AlCl₃</th>
<th>+AlCl₃ + HCl</th>
</tr>
</thead>
</table>

**IR (ν max., cm⁻¹, KBr)**

3300 br, 1710, 1640, 1450, 1325, 1200, 1010, 860, 850, 840 and 750.

Rf: See Table 3
Acid hydrolysis of compound E (Luteolin, D-glucose)

Compound E (10 mg) in 2 N HCl at 100° for 3 h yielded luteolin and D-glucose identified by co-PC with authentic samples. The aglycone-sugar ratio was found to be 1:1.

Enzyme hydrolysis of Compound E (Luteolin, D-glucose)

Compound E (5 mg) on treatment with 2 ml acetate buffer (pH: 5) and 1 mg of β-glucosidase at 38° for 24 h yielded luteolin and D-glucose.
Fig. 3

$^1\text{H NMR SPECTRUM OF BRACTEIN}$ (See page 75)
Fig. 5

$^1$H NMR SPECTRUM OF CERNUOSIDE (See page 78)
MASS SPECTRUM: 2
SAMPLE: AU2, DR AGR NAIR
NOTE: 1/6/80
R.T.: 8:00 - TIM 1.9, REL. 937.6
BASE PEAK: M/E 265.0, INT. 959.9

Fig. 6
MASS SPECTRUM OF AUREUSIDIN (See page 80)
Table 1

Column Chromatography of the crude extract of

Pink Flowers of H. bracteatum

<table>
<thead>
<tr>
<th>Weight of the residue</th>
<th>= 3.5 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of silica gel mixed with residue</td>
<td>= 20 g</td>
</tr>
<tr>
<td>Weight of silica gel used in column</td>
<td>= 100 g</td>
</tr>
<tr>
<td>Volume of each fraction collected</td>
<td>= 100 ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Eluting solvent</th>
<th>Fraction number</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCl₃</td>
<td>1-3</td>
<td>No UV fluorescent spot</td>
</tr>
<tr>
<td>CHCl₃: MeOH (9:1)</td>
<td>4-8</td>
<td>Intense yellow residue, mixture of four compounds</td>
</tr>
<tr>
<td>CHCl₃: MeOH (3:1)</td>
<td>9-15</td>
<td>'</td>
</tr>
<tr>
<td>CHCl₃: MeOH (1:1)</td>
<td>16-20</td>
<td>'</td>
</tr>
<tr>
<td>CHCl₃: MeOH (1:3)</td>
<td>21-30</td>
<td>Pale yellow solid, homogeneous.</td>
</tr>
<tr>
<td>CHCl₃: MeOH (1:9)</td>
<td>31-35</td>
<td>'</td>
</tr>
<tr>
<td>MeOH</td>
<td>36-40</td>
<td>No characteristic spot.</td>
</tr>
</tbody>
</table>
### Table 2

**Column Chromatography of EtOAc and MeCOEt extract of Ivory White Flowers of H. bracteatum**

<table>
<thead>
<tr>
<th>Eluting solvent</th>
<th>Fraction number</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCl₃</td>
<td>1-3</td>
<td>No UV fluorescent spot</td>
</tr>
<tr>
<td>CHCl₃: MeOH (3:1)</td>
<td>4-6</td>
<td>Yellow solid, homogeneous</td>
</tr>
<tr>
<td>CHCl₃: MeOH (1:1)</td>
<td>7-10</td>
<td>&quot;</td>
</tr>
<tr>
<td>CHCl₃: MeOH (1:3)</td>
<td>11-15</td>
<td>Pale Yellow Solid, homogeneous</td>
</tr>
<tr>
<td>MeOH</td>
<td>16-18</td>
<td>No characteristic spot.</td>
</tr>
</tbody>
</table>
### Table 3

**Rf Values of the Polyphenolics of H. bractiatum**

*Rf x 100 (Whatman No.1, ascending, 28 ± 2°)*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Developing Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distilled H₂O</td>
</tr>
<tr>
<td>Bractein</td>
<td>7</td>
</tr>
<tr>
<td>Bracteatin</td>
<td>0</td>
</tr>
<tr>
<td>Cernuoside</td>
<td>10</td>
</tr>
<tr>
<td>Aureusidin</td>
<td>0</td>
</tr>
<tr>
<td>Luteolin</td>
<td>0</td>
</tr>
<tr>
<td>Iso-orientin</td>
<td>23</td>
</tr>
<tr>
<td>Luteolin-7-O glucoside</td>
<td>10</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>66</td>
</tr>
</tbody>
</table>
Table 4

**Rf Values of Sugar Obtained by Hydrolysis of Flavones and Aurone Glycosides of *H. bracteatum***

Rf x 100 (Whatman No.1, ascending, 28 + 2°)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Developing Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n-BuOH: Water t-BuOH: EtOAc n-BuOH C₆H₅O₃H:</td>
</tr>
<tr>
<td>HOAc:</td>
<td>satd. HOAc: Pyrid- EtOH: n-BuOH</td>
</tr>
<tr>
<td>H₂O</td>
<td>Phenol H₂O ine H₂O Pyrid-</td>
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<tr>
<td>phenol</td>
<td>(2:1:5, (3:1:1) H₂O (4:1:4) ine</td>
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<td>upper)</td>
<td>t-BAW (10:4:3) BEW H₂O</td>
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<tr>
<td>BAW</td>
<td>EPW (1:5:3:3) BBPW</td>
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* Spotted for comparison.
REFERENCES


