Chapter -IV

Chemical Examination of Flowers of

*Tagetes erecta*

- Introduction
- Experimental
- Results and Discussion
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INTRODUCTION

Taxonomy and distribution

The family Asteraceae is one of the largest family, which includes about 1100 genera and 30,000 species. About 157 genera and 900 species are reported from India. *Tagetes erecta* (Gainda) belongs to family Asteraceae, is an annual, sparsely-branched, aromatic herb. Branches angular, ribbed. Leaves pinnate, oblong, acute, base decurrent. Flowers yellow or orange. Flowering throughout the year. It is most commonly cultivated in gardens in major parts of India [1]. The pastes of flowers were often applied on wound and cuts and leaves juice dropped in otalgia [1].

Economic importance

Most of the species of *Tagetes* were analysed for their essential oil compositions [2]. *Tagetes patula* is widely distributed in montane and sub montane Himalayan zone, Its leaves powder is used as an insect repellent and paste used in skin ailments [1]. Flowers of *Tagetes erecta* are richest source of yellow dyes in ancient arts of Rajasthan and Orrisa [3].

Principle constituents isolated from flowers of *T. minuta* are anthocyanins and it derivatives [4]. Some long chain fatty acids, aromatic hydrocarbons and phenyl acetaldehydes are extracted from floral extract of *T. erecta*. On the basis of thorough survey of literature, it has been found that no more phytochemical, dyes and dyeing studies have been reported in this genus. Therefore, it was selected for studies.
Experimental

Collection of plant material

The flowers of *Tagetes erecta* was collected from the Pasulok, Rishikesh of District Dehradun, Garhwal in the month of September 2005. The identity of the plant was confirmed by Dr. P.K. Uniyal, Department of Botany, H.N.B. Garhwal University Campus, Badshahithaul, Tehri Garhwal (U.A.) and the voucher specimen is available in the herbarium of Plant Identification Laboratory of Botany Department.

Extraction and isolation

The air-dried and coarsely powdered flowers of the plant were defatted with light petroleum in a soxhlet. The defatted mass was exhaustively extracted repeatedly with 90 % aqueous EtOH, untill the extractive became colourless. All the extracts were mixed and concentrated under reduced pressure using rotatory vacuum evaporator.

The concentrated extract was adsorbed on Silica gel and fractionated through column chromatography using the solvent system of chloroform-methanol (95:5). The polarity of solvent was gradually increased by addition of methanol. Repeated column chromatography afforded compounds MT-1, MT-2, MT-3, MT-4, MT-5, MT-6 and MT-7 with some other inseparable compounds.
Results and Discussion

MT-1

It was crystallized from methanol as white needles.

Melting Point : 135-137 °C
Molecular Formula : C_{29}H_{50}O
Molecular Weight : 414 amu
I.R. (ν_{max}^{KBr}) cm^{-1} : 3340, 2970, 2959, 2920, 1640, 1463.
EI-MS (m/z) : 414 [M]^+, 412, 394, 381, 300, 273, 255, 213.

Elemental analysis of compound corresponded to the molecular formula C_{29}H_{50}O compound MH-1 was found to be a sterol, as it responded positive to Liebermann-Burchard and Noller’s tests [5]. It also responded positive to TNM test for unsaturation. Its negative test with Molisch’s reagent indicated the non-glycosidic nature of the compound. The IR spectrum of compound showed characteristic absorption at 3340 cm^{-1} (CH stretching) and 1640 for (C=C stretching). On the basis of above facts compound MH-1 was identified as β-Sitosterol (Figure-1). It was further confirmed by co-TLC, co-IR and mmp with an authentic sample [6].

![Figure-1](image-url)
MT-2

It was crystallized from methanol as yellow solid.

**Melting Point**: 160-165 °C

**Molecular Formula**: $C_{21}H_{21}O_9$

**Molecular Weight**: 417 amu

**FAB-MS (m/z)**: $439[M+Na]^+$, $417[M]^+$, 255 $[M-162]^+$

**$^1$H-NMR (CH$_3$OD, $\delta$ppm)**: 2.5(s,CH$_3$, H-3), 7.26(brs, H-2), 7.55 (brs,H4), 7.95(m,H5), 7.8(m,H7), 5.30(d, J=8.0Hz, H-1’of galactose)

**$^{13}$C-NMR (CH$_3$OD, $\delta$ppm)**: 136(C-1), 147(C-2), 123(C-3), 124(C-4), 131(C-5), 161(C-6), 114(C-7), 158(C-8), 119(C-9), 178(C-10), 134(C-11), 181(C-12), 120(C-13), 21.54(-CH$_3$).

**Galactopyranosyl (C$_1$ - C$_6$)**: 101.34, 73.57, 77.5, 69.9, 76.49, 61.04.

It was crystallized from methanol as yellow crystalline solid. It gave positive test for anthraquinone. On acidic hydrolysis compound afforded an aglycone, M.P. 195-196 °C identified as 1, 8-dihydroxy 3-methyl anthraquinone (by mmp, co TLC). The neutralized hydrolysate on PC showed the presence of galactose (authentic sample run parallel). The molecular mass of compound was deduced 417 amu from its FAB-MS (Figure-2), which furnishes the peaks at m/z $439[M+Na]^+$, $417[M]^+$, 255$[M-162]^+$, suggesting the loss of hexosyl (galactosyl) unit from molecular ion peak. In order to look into the structure of compound it was methylated by Hokomorie’s method [7]. The aglycone obtained by HOH of above methylated product was identified as 1, 8-dihydroxy 3-methyl
anthraquinone. This established the attachment of sugar moiety at position 8 -of aglycone. The hydrolysate on PC showed the presence of 2, 3, 4, 6-tetra-O-methyl-D-galactose.

In $^1$H-NMR spectrum (Figure-3a,) of compound showed a doublet at $\delta$ 5.30(d, J=8.0Hz) was assigned for anomeric protons (1H-of glycosidic moiety). The type of linkage was further assigned by $^1$H, $^{13}$C-NMR (Figure-3b) data. Thus these spectral finding confirmed the identity of compound MT-2 Crysophenol 8-O-β-D-galactopyranoside (Figure-4)
MT-3

It was crystallized from methanol as crystalline solid.

**Melting Point** :  227-229 °C

**Molecular Formula** :  C_{26}H_{28}O_{14}

**Molecular Weight** :  564 amu

**IR (ν_{max}^{KBr}) cm^{-1}** :  3240, 3040, 1895, 1660, 1450, 1190, 915, 850.

**UV (λ_{max}^{MeOH}) nm** :  222, 254, 280, 410.

**FAB-MS (m/z)** :  564[M]^+, 402[M-162]^+, 208[M-162+132]^+

^{1}H-NMR (C_{5}D_{5}N, δ ppm) :

3.80 (3H, s, CH3),  7.55 (d, J=8.0Hz, H-3),  6.95 (dd, J=7.8,1.8Hz, H-7),  7.76 (dd, J=7.6,1.8  Hz, H-5),  7.28 (d, J=1.2Hz, H),  5.8 (d, J=7.2Hz, H-1’),  3.19 (m),  3.37 (m),  3.46 (m),  3.63 (dd, J=5.6Hz, H-6’, glucose),  4.62 (d, J=2.0Hz, H-1’’), xylose).

^{13}C-NMR (C_{5}D_{5}N, δ ppm)

**Aglycone**

142.2 (C-1), 142.8 (C-2), 124.2 (C-3), 124.6 (C-4), 112.7 (C-5), 151.2 (C-6), 123.2 (C-7), 158.1 (C-8), 80.23 (C-9), 179.2 (C-10), 139.0 (C-11), 133.3 (C-12), 114.1 (C-13), 139.8 (C-14) 29.9 (CH3).

**Glycone**

**Glucose** :  104.8 (C-1’’), 74.5 (C-2’’), 77.6 (C-3’’), 71.0 (C-4’’), 7.0 (C-5’’), 62.3 (C-6’’).

**Xylose** :  102, 9 (C-1’’’), 74.7 (C-2’’’), 74.9 (C-3’’’), 71.8 (C-4’’’), 61.9 (C-5’’’).
It was crystallized from methanol as crystalline solid. On the basis of elemental analysis and molecular weight determination its molecular formula was deduced as C_{26}H_{28}O_{14}. The molecular weight of compound was found to be 564[M]^+ from FAB-MS. The fragment peaks at m/z 402 and 208 are arose by loss of one hexose and one pentose units from molecular ion peak. The UV spectrum showed absorption at 218, 225, 305 and 315. Its IR spectrum displayed a characteristic band of chelated carbonyl group at 1625 cm^{-1}. The $^{13}$C-NMR spectrum of compound showed the presence of 26 carbon atoms. The assignment of all proton and carbon were achieved by $^1$H-$^1$H-HOMO(COSY) and inverse $^{13}$C-NMR data, which showed the anthraquinone skeleton of compound. The anomeric proton of compound exhibit doublet at $\delta$ 5.89(J=7.2Hz) anomeric proton of $\beta$-linked D-glucose. A doublet at $\delta$ 4.62(J=2.8Hz) were assigned for $\alpha$-linked xylose. In $^{13}$C-NMR of compound MT-3, C-6 of glucose showed downfield shift at $\delta$ 62.32 while a slightly shielded C-5 resonate at $\delta$ 77.06 assigned for glycosidic linkage at C-6 of glucose. The downfield signal at $\delta$ 142.2, 142.8, 115.1, 153.18 were assigned for substitution at C-1, C-2, C-6 and C-8 carbon atoms, C-8 found to be glycosilated. Thus on the basis of above data MT-3 was identified as Anthraquinone 1,6-dihydroxy 2-methyl 8-O-$\beta$-D-glucopyranosyl (1$\rightarrow$6)-$\alpha$-L-xylopyranoside (Figure-5).
MT-4

It was crystallized form methanol as fine needles.

Melting Point : 190-195 °C
Molecular Formula : C₁₂ H₁₆ O₇
Molecular Weight : 272 amu
FAB-MS (m/z) : 272 [M]+, 110 [M-162]+

1H-NMR (C₅ D₅ N, 400MHz, δ ppm):
Aglycone : 9.00(1H.s,OH), 6.86(1H,d, J=8.8Hz,H2,6), 6.64 (2H, d, J=8.8Hz, H-3,5)
Glycone : 4.62(1H,d,J=7.3Hz,H-1’),3.66(1H, dd, J=7 3Hz, H-2’), 3.15-3.45 (m, sugar protons, H- 3’, 4’, 5’, 6’).

13C-NMR (C₅ D₅ N, 100MHz, δ ppm):
Aglycone : 152.2 (C-1), 117.7(C-3, 5) 115.5 (C-2, 6), 150.4 (C-4).
Glycone : 101.7 (C-1’), 76.7 (C-2’), 76.6 (C-3’), 73.3 (C-4’), 69.8 (C-5’), 60.8 (C-6’).

Elution of column with chloroform methanol (95:5) afforded a compound MT-4. It gave negative Libermann-Burchard test and was crystallized from methanol as fine needles. On acidic hydrolysis with 7% HCl it gave an aglycone, which was identified as hydroquinone (by comparing with an authentic sample). The sugar moiety was also identified as β-D-glucose (by paper chromatography). The 1H-NMR spectrum (Figure-6,7) of compound showed A2 B2 type doublet 2 each integrating for two protons at δ 6.85 (J=8.8Hz) and δ 6.64 (J=8.8Hz) were
assigned for H-2 and H-6 protons of aromatic ring while a singlet at $\delta$ 9.00 was assigned to OH group present in the compound.

The $^{13}$C-NMR spectra (Figure-8) of compound showed aromatic signals for benzene ring in the downfield region at $\delta$ 152.2(C-1), 117.7(C-3, 5), 115.5(C-2) and 150.4(C-4).

A doublet at $\delta$ 4.91(d, J=8.0Hz) was attributed for the anomeric proton of D-glucose unit while other protons of glucose moiety were found to appear at $\delta$ 3.70-3.48.

On permethylation with NaH and CH$_3$I by Hakomori’s method [7] it afforded 2, 3, 4, 6-terra-O methyl-D-glucose. FAB-MS and ES-MS spectrum (Figure-9, 10) showed molecular weight of compound to be 272. The peak at $m/z$ 110 was due to the loss of hexose unit. These peaks showed that a glycone was glycosylated with glucose, which is $\beta$-linked as shown by $^1$H-NMR.

Thus on the basis of above studies compound was characterized as Hydroquinone-1-O- $\beta$-D-glucopyranoside (Figure-11).
MT-5

It was crystallize from methanol.

**Melting Point**: 325-327 °C

**Molecular Formula**: C_{12}H_{16}O_{8}

**Molecular Weight**: 288 amu

{\textsuperscript{1}H-NMR (CH\textsubscript{3}OD, 400MHz δ ppm)}:

7.17(1H, d, J=8.0Hz, H-5), 7.19(1H, d J=8.0Hz, H-6), 1.29(3H, s, CH\textsubscript{3}), 5.03 (1H, J=4Hz, H-1) (anomeric proton), 3.31-3.9(m, sugar protons).

{\textsuperscript{13}C-NMR (CH\textsubscript{3}OD, 100MHz, δ ppm)}:

132.6(C-2), 163.0(C-3), 199.4(C-4), 131.6(C-5), 117.2(C-6), 2641(-CH\textsubscript{3}), 101.6(C-1), 74.9(C-2’), 77.9(C-3’), 71.2(C-4’), 78.3(C-5’), 62.3(C-6’).

It was crystallized from methanol as fine crystalline powder. It gave positive test with Molish reagent there by indicating the glycosidic nature of the compound. The IR spectrum displayed the characteristic peaks at 1655 and 1612 cm\textsuperscript{-1}, which showed the presence of carbonyl function and aromatic system in the compound. \textsuperscript{1}H-NMR spectrum (Figure-12,13,14) of the compound showed two separate doublets each integration for one proton at δ 7.17 (J=8.0Hz) and δ 7.19(J=8.0Hz) were attributed to the C-5 and C-6, while a singlet appeared at δ 1.29 was assigned for a methyl group present in the compound E. Tee \textsuperscript{1}H-NMR spectrum further shows a doublet at δ 5.03 (J=4Hz) was assigned to the anomaric proton H-1’ of the glycosidic moiety. The other sugar protons were found to appear in between 3.31-3.92 in \textsuperscript{1}H-NMR spectrum.

The \textsuperscript{13}C-NMR spectrum (Figure- 15,16,17) of compound show presence of 12 carbon atoms. The downfield peak at δ 199.4 in \textsuperscript{13}C-NMR
spectrum indicating the presence of $\alpha$, $\beta$ unsaturated carbonyl function present in the compound, while C-5 and C-6 were found to be appeared at $\delta$ 131.6 and $\delta$ 117.2.

Thus on the basis of above observations compound was identified as 2-methyl pyran-3-O- $\beta$- D-glucoside (Figure- 18).

![Figure- 18](image)

**Acidic Hydrolysis of Compound**

Compound (10 mg) was hydrolyzed with 6% $\text{H}_2\text{SO}_4$ for hours. The reaction mixture was cooled and poured in ice water and then fractionated with ethyl acetate (2 x 20ml). The organic layer was dried over anhydrous sodium sulphate. The solvent was removed and the aglycone was crystallized as solid, (8 mg). The aqueous layer was neutralized with sodium bicarbonate and was analysed by paper chromatography and the sugar moiety was identified as glucose with authentic sample by using n-BuOH: ACOH: $\text{H}_2\text{O}$ (4: 1:5) solvent system.
MT-6

It was crystallized as brownish yellow powder from chloroform.

**Melting Point**: 174-176 °C

**Molecular Formula**: C\textsubscript{30}H\textsubscript{36}O\textsubscript{15}

**Molecular Weight**: 636 amu

**IR (ν\textsubscript{max} \textsuperscript{KBr}) cm\textsuperscript{-1}**: 3340, 1642

**FAB-MS (m/z)**:

- 637 (M+H)\textsuperscript{+}, 636 (M)\textsuperscript{+}, 490(M-146)\textsuperscript{+}, 328 (M-(146+162))\textsuperscript{+}, 307[M-(146+162+18)]\textsuperscript{+}, 232[M-(146+162+2x18+59)]\textsuperscript{+}.

\textsuperscript{1}H-NMR (DMSO, δ ppm):

- 7.05(d, J=1.2Hz, β 2H), 6.36(d, J=1.2Hz, H-3), 7.6(d, J=2.5Hz H-5), 6.7(d, J=2.5Hz, H-6), 7.2(s,H-2’), 6.9(5,H-6’) 3.25(5,acetyl), 1.24(3H), (OCH\textsubscript{3}), 0.8 (3H, 6’’’), 5.8(d, J=6.8Hz) (anomeric proton 1’’’) 4.0 (s, 1’’’).

\textsuperscript{13}C-NMR (DMSO, δ ppm):

<table>
<thead>
<tr>
<th>Carbon No. (aglycone)</th>
<th>Chemical Shift (δ ppm)</th>
<th>Carbon No. (aglycone)</th>
<th>Chemical Shift (δ ppm)</th>
<th>Chemical shift of Sugar</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td></td>
<td>Sugar</td>
</tr>
<tr>
<td>C-1</td>
<td>105.6</td>
<td>C-1’</td>
<td>123.2</td>
<td>C-1’’’</td>
</tr>
<tr>
<td>C-4</td>
<td>176.4</td>
<td>C-2’</td>
<td>113.6</td>
<td>C-2’’’</td>
</tr>
<tr>
<td>C-5</td>
<td>125.4</td>
<td>C-3’</td>
<td>163.3</td>
<td>C-3’’’</td>
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<tr>
<td>C-6</td>
<td>116.0</td>
<td>C-4’</td>
<td>149.0</td>
<td>C-4’’’</td>
</tr>
<tr>
<td>C-7</td>
<td>165.0</td>
<td>C-5’</td>
<td>148.3</td>
<td>C-5’’’</td>
</tr>
<tr>
<td>C-8</td>
<td>117.3</td>
<td>C-6’</td>
<td>112.2</td>
<td>C-6’’’</td>
</tr>
<tr>
<td>C-9</td>
<td>117.5</td>
<td>C=O</td>
<td>169.3</td>
<td></td>
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<tr>
<td>C-10</td>
<td>17.5</td>
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|                      |                        |                      |                        | Rhamnose              |
|                      |                        |                      |                        | C-1’’’                 |
|                      |                        |                      |                        | C-2’’’                 |
|                      |                        |                      |                        | C-3’’’                 |
|                      |                        |                      |                        | C-4’’’                 |
|                      |                        |                      |                        | C-5’’’                 |
|                      |                        |                      |                        | C-6’’’                 |
It was crystallized from chloroform as yellow brown powder. On the basis of elemental analysis and molecular weight determination its molecular formula was deduced as $C_{30}H_{36}O_{15}$. It showed positive test with FeCl$_3$, Mg/HCl and Molisch’s reagent. Its IR spectrum displayed a absorption bands at 3340 cm$^{-1}$ and 1642 cm$^{-1}$ characteristic of hydroxyl and carbonyl groups [8] in molecule. The FAB-MS (Figure-19) of compound displayed ion peak at $m/z$ 490[M-146]$^+$, 8[M-146+162]$^+$, 360[M-146+162+H$_2$O]$^+$, 289[M-146+162+2x H$_2$O]$^+$, 232[M-146+162+2xH$_2$O+OCOCH$_3$]$^+$, thereby indicating the diglycoside nature of compound. $^1$H-NMR spectrum of compound showed three doublets at $\delta$ 6.36(d, J=1.2Hz), 7.6(d, J=2.5Hz) and 6.7(d, J=2.5Hz) assigned for 3H, 5H and 6H protons. The position of two singlets at $\delta$ 7.2 and 6.9 were further in agreement with H-2’ and H-6’ protons. The upfield sharp singlets at $\delta$ 3.26 were assigned for acetyl and a singlet at $\delta$ 1.24 for chalcone methyl group. The proton multiplet between $\delta$ 3.4-4.0 represent the position of sugar proton, whereas a singlet at 0.8 and 4.0 showed the position of $\alpha$-linked rhamnose. The appearance of doublet at $\delta$ 5.8(d, J=6.8Hz) further confirm the position $\beta$-linked anomeric proton of hexose. The $^{13}$C-NMR spectrum (Figure-20) of compound showed thirty carbon peaks resonated at aromatic and carbohydrate region. The downfield peak at $\delta$ 176.4 confirm the present of carbonyl function, whereas the peaks at 165.0(C-7), 163.3(C-3), 149.2(C-4’) and 148.3(C-5’) substitution at these positions. The appearance of peak at $\delta$ 169.0 was assigned for the acetyl carbon atom. Compound MT-6 on acidic hydrolysis (7% MeOH/HCl) afforded glycone and aglycone. The concentrated and neutralized aglycone on PC showed presence of rhamnose and glucose (Rf values). The aglycone was identified as 3’ acetyl 4’, 5’, 7’ tri-hydroxy chalcone (comparison of $^{13}$C, 1H-NMR data with reported compounds). Partial HOH (5% MeOH+HCl) showed the loss of rhamnose (PC, Rf values). This further showed the linear attachment of sugar with aglycone.
Thus on the basis of above spectral studies the compound MT-6 was identified as Chalcone 3’-acetyl 4’, 5’ di-hydroxy-7-[O-β-D-glucopyranosyl (1→4)] α-L rhamnopyranosyl (Figure- 21).
MT-7

It was crystallized from chloroform and as yellow powder.

Melting Point : 177-178 ºC
Molecular Formula : C_{16}H_{12}O_{6}
Molecular Weight : 300 amu
I.R (ν_{max}^KBr) cm\(^{-1}\) : 3450(OH), 2925, 1662(C=O), 1630 (chelated C=O), 1580, 1517, 1320, 1180, 1060, 772, 620.
EI-MS (m/z) : 300[M]^+, 281, 257, 229, 212, 186, 155, 136

\(^1\)H-NMR (DMSO d6, δ ppm) :
12.9(brs,1-OH), 6.54(d,J=2.2Hz,H-2), 7.16(d,J=2.2Hz,H-4), 7.57(s,H-5), 3.98(s,OCH\(_3\)), 4.02(s,OCH\(_3\)), 7.64(s,H-8).

\(^13\)C-NMR (DMSO d6, δ ppm) :
166.8(C-1), 105.2(C-2), 164.6(C-3), 107.3(C-4), 111.3(C-5), 153.3(C-6), 153.5(C-7), 108.2(C-8), 184.5(C-9), 181.2(C-10), 127.8(C-11), 127.5(C-12), 108.4(C-13), 134.8 (C-14), 56.1(OCH\(_3\)), 56.1(OCH\(_3\)).

It was crystallized from chloroform as yellow powder. It showed IR bands at 3450, 1662 and 1630 cm\(^{-1}\) for free hydroxyl, unchelated and chelated carbonyl groups, respectively. The EI mass spectrum of compound displayed the peak at m/z 300 [M]^+, calculated for molecular formula C_{16}H_{12}O_{6}. The \(^1\)H-NMR spectrum of compound showed a peri hydroxyl group at δ12.9 and two meta–coupled protons at δ 6.55(d,J=2.2Hz,H-2) and 7.17(d,J=2.2Hz,H-4). However, singlets for two methoxyl group at δ 3.98(s) and δ 4.01(s) and also two downfield shifted protons at δ 7.59 and δ 7.64 were assigned for H-5 and H-8 protons of compound MT-7.
The $^{13}$C-NMR data of the compound indicate the presence of two equivalent methoxy groups, and one unchelated and chelated carbonyl carbons appeared at δ 56.1, 181.2 and 184.5. Two dimensional NOESY cross peak correlation were observed between the two methoxy proton at δ 3.98 and δ 4.01 and the two one proton singlets at δ 7.59(H-5) and δ 7.64(H-8) respectively.

Thus on the basis of above spectral findings the compound MT-7 was identified as 6,7-dimethoxy xanthopurpurin (Figure-22). It was further confirmed by comparison of its data with that of reported compound 6-hydroxy xanthopurpurin [9].
References