Chapter 4

Chemical studies of the rhizomes of *Curcuma aromatica* Salisb.
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4.1. Introduction

*Curcuma aromatica* Salisb. (Zingiberaceae) is an erect perennial herb cultivated throughout India. This plant is commonly known as Jangali Haldi (wild turmeric). The rhizomes are tuberous, large, orange-red and aromatic and the fresh root has a camphoraceous odour. They are used in combination with astringents and aromatics for bruises, sprains, hiccough, bronchitis, cough, leucoderma and skin eruptions.\(^1\) They are also widely used as a flavouring agent, condiment, and a source of yellow dye, and possess strong antioxidant and antimicrobial properties.\(^2\)\(^-\)\(^4\) The dried rhizome is used as a carminative.\(^5\) Rhizomes yield 6.1% essential oil.\(^6\) Oil is used for treatment of early stage of cervix cancer.\(^7\) The major constituents of the leaf oil were found to be camphor (28.5%), ar-turmerone (13.2%), curzerenone (6.2%), 1,8-cineole (6.0%) and a-turmerone (2.5%). The rhizome oil contained mainly of camphor (32.3%), curzerenone (11.0%), a-turmerone (6.7%), ar-turmerone (6.3%) and 1,8-cineole (5.5%).\(^8\)\(^,\)\(^9\) Many sesquiterpenes - isozedoarondiol, methylzedoarondiol, neocurdione, germacrone, curdione, \((4S,5S)\)-germacrone4,5-epoxide, dehydrocurdione, procurcumenol, zedoarondiol and curcumenone were isolated from *Curcuma aromatica* Salisb.\(^10\),\(^11\) Neocurdione, isoprocucumenol and 9-oxo-neoprocurcumenol were isolated from fresh rhizomes of *Curcuma aromatica*.\(^12\)
Curcumin, demethoxycurcumin and β-sitosterol-3-O-β-D-glucopyranoside have been also isolated from the ethyl acetate extract of rhizomes of this plant.\cite{13}

Phan et al. also reported the isolation of sesquiterpenes hydrocarbons, α-humulene, β-selinene and α-selinene along with sesquiterpenoids.\cite{14,15} The colouring matter of this plant is curcumin. Curcuminoids showed anti-cancer activity.\cite{16} Madhu et al. reported the isolation of two guanine type sisqueterpenes, 9-oxoneoprocurcumenol and neoprocurcumenol. 9-oxoneoprocurcumenol exerted significant toxicity on mosquito larvae.\cite{17}

Herein, the isolation and identification of compounds from this plant are reported. Further the transformations of the bioactive compounds by semi-synthesis are discussed.

4.2. Results and Discussion

The chloroform extract of *C. aromatica* was fractionated by column chromatography and the fractions were purified by repeated column chromatography to give the compounds, 1-6 (Figure 1).

![Figure 1. Structures of compounds 1-6](image-url)
The structures of zedoaronediol (1), Isozedoarondiol (2), curcumenol (3) and zederone (4) were confirmed by comparing with the published data.\textsuperscript{10-15}

The structure of 6 was determined by its IR, \textsuperscript{1}H NMR, \textsuperscript{13}C NMR spectral data. Compound 6, white solid, was isolated from the chloroform extract by elution with PE:EA, 4:1 v/v and was found to be a steroid indicated by Libermann Burchard (LB) test, C\textsubscript{40}H\textsubscript{62}O\textsubscript{10}. IR spectrum of 6 (figure 2) showed characteristic absorption bands at 3429, 3057, 1543, 1425, 1258, 1155, 858, 739 cm\textsuperscript{-1}. The \textsuperscript{1}H-NMR (400MHZ, DMSO-d\textsubscript{6}) spectrum (figure 3) showed the presence of six methyl groups at δ 0.64, 0.75, 0.81, 0.88, 0.92 and 1.12; presence of one olefinic proton was evident at δ 5.31 (1H, m). The signal at C-3 showing multiplet (seven peaks) was found at δ 3.32. From the analysis of \textsuperscript{13}C-NMR (100MHz, DMSO-d\textsubscript{6}) spectrum (figures 4-6) with DEPT spectra (figures 7-11), 6 showed twenty nine carbon signals including 6×CH\textsubscript{3}, 12×CH\textsubscript{2}, 14×CH and 3×C. On the basis of physical constant, IR, \textsuperscript{1}H-NMR, \textsuperscript{13}C-NMR with DEPT spectral data; and by comparing with the authentic sample, 6 was established as β-sitosterol-α-D-glucoside.
Figure 2. IR spectrum of compound 6.
Figure 3. $^1$H NMR spectrum of compound 6
Figure 4. $^{13}$C NMR spectrum of compound 6
Figure 5. $^{13}$C NMR spectrum of compound 6

Figure 6. $^{13}$C NMR spectrum of compound 6
Figure 7. $^{13}$C DEPT-45 NMR spectrum of compound 6

Figure 8. $^{13}$C DEPT-45 NMR spectrum of compound 6
Figure 9. $^{13}$C DEPT-90 NMR spectrum of compound 6

Figure 10. $^{13}$C DEPT-135 NMR spectrum of compound 6
Figure 11. $^{13}$C DEPT-135 NMR spectrum of compound 6

Figure 12. $^1$H-$^1$H COSY spectrum of compound 6
Figure 13. $^1$H-$^1$H COSY spectrum of compound 6

Figure 14. Mass spectrum of compound 6
4.2.1. Transformative reactions of Zederone

Sesquiterpenoids represent a varied class of natural products. Thousands of structures have been isolated and reported with hundreds of new derivatives discovering each year. It is interested to introduce some groups or atoms to sesquiterpenoids through transformative reactions. There exists an ample opportunity to study the transformative reactions on the isolated sesquiterpenoids using various reagents, and also the biological activities of each of them in comparison to the parent compound. It has been attempted on the transformative reactions of zederone, a sesquiterpenoid isolated from *C. aromatica*.

The carbonyl group of zederone 4 was easily reduced by treatment with sodium borohydride (NaBH₄) at 0°C to generate zederol 7. The conversion of zederone to zederol, can be confirmed by comparing the IR spectra of 4 and 7 (figure 15). The IR spectrum of 7 showed a hydroxyl peak at 3440 cm⁻¹, which was not observed in the IR spectrum of 4. Further, the carbonyl peak of zederone (1662 cm⁻¹) was not observed in the IR spectrum of zederol (figure 16). The ¹H NMR spectrum (figure 17) of 7 showed a singlet at δ₇ 2.97 (1H, s, 6-H) and the hydroxyl proton at δ₇ 7.24 which were not observed in the ¹H NMR spectrum of 4. Compound 7 was obtained as a crystalline solid, m.p.152-154°C, and the molecular formula was determined as C₁₅H₁₉O₃ by the presence of a pseudomolcular ion [M+H]⁺ at m/z 248.92 in its TOF-MS (figure 18). Thus the transformation of zederone to zederol by reduction with sodium borohydride takes place (Scheme 1).
Figure 15. Comparison of FT-IR spectra of zederone, 4 and zederol, 7

Figure 16. IR spectrum of zederol, 7
Figure 17. $^1$H NMR Spectrum of compound 7

Figure 18. Mass spectrum of compound 7
When zederol 7 was treated with ethyl acetate, it afforded a lactone 8. Its IR spectrum (figure 19) displayed peaks at 3418 cm$^{-1}$ of hydroxyl group and 1751 cm$^{-1}$ due to carbonyl group of the lactone ring. The final confirmation of the structure could be done by taking NMR and Mass spectral data. Further reaction of this compound 8 with N-bromosuccinimide yielded a bromination product 9, whose IR spectrum (figure 20) showed peaks at 3462 cm$^{-1}$ (OH) and 1711 cm$^{-1}$ (CO). However, when zederone (4) itself is reacted with NBS in presence of dimethyl sulphide afforded a bromination product 10 whose IR spectrum is given in figure 21. But zederol (7) on bromination with NBS in dimethyl sulphide undergo rearrangement to yield a saturated cyclic ketone 11 (figure 22). The overall transformative reactions of zederone into various derivatives is given in scheme1.

![Figure 19. IR spectrum of compound 8](image-url)
Figure 20. IR spectrum of compound 9

Figure 21. IR spectrum of compound 10
Figure 22. IR spectrum of compound 11

Scheme 1. Preparation of Zederone Derivatives

It was attempted to react zederone (4) with hydrazine in presence of ethanol. But instead of forming the corresponding hydrazone or cyclised product,
ring opening of the epoxide was taken to give an \( \alpha,\beta \)-unsaturated ketone 12 (scheme 2). The IR spectrum of 12 (figure 23) showed a peak at 1668 cm\(^{-1}\) due to the unsaturated carbonyl group. Its \( ^1 \)HNMR spectrum (figure 24) revealed a signal at \( \delta_H \) 7.27 (s, 1H, H-5).

![Figure 22. IR spectrum of compound 12](image)

![Figure 24. \( ^1 \)H NMR spectrum of compound 12](image)
Similarly, the reaction of zederone 4 with phenylhydrazine in ethanol afforded the compound 13 due to attack at epoxy ring. The IR spectrum of 13 showed a peaks at 3396, 3293, 1659 cm\(^{-1}\) due to -OH, -NH, -CO groups respectively (Figure 25). The structure of compound 12 may be confirmed by NMR and mass spectral data.

![Figure 25. IR spectrum of compound 13](image)

**Scheme 2. Preparation of Zederone Derivatives**

**4.3. Experimental**

**4.3.1. General Procedure**

Melting points were measured on a melting point apparatus (BUCHI) and are uncorrected. Optical rotations were measured on an Autopol II: Rudolph Research Analytical, Automatic Polarimeter 30415 equipped with a sodium lamp (589 nm) and a 10 cm microcell. Silica gel 60-120 mesh (Merck) were used for
column chromatography eluted with petroleum ether with an increasing ratio of ethyl acetate. The spots of TLC were detected by spraying LB reagent, followed by heating. IR spectra (Shimazdu IR-408 spectrometer) were recorded in wave numbers (cm$^{-1}$); $^1$H NMR (Bruker AC-400 MHz spectrometer) and $^{13}$C NMR spectra (Bruker AC-100 MHz spectrometer) were recorded using residual non-deuterated solvent as an internal reference and all chemical shifts ($\delta_H$ and $\delta_C$) are quoted in parts per million (ppm) downfield from tetramethylsilane (TMS); Mass spectra were recorded by Water ZQ-4000 and Jeol-D 300 mass spectrometer.

4.3.2. Plant materials

The rhizomes of *Curcuma aromatica* Salisb. were collected from Thoubal district of Manipur. This plant has been deposited in the Department of Life Sciences, Manipur University, Imphal.

![Image](image_url)

Figure 26. *Curcuma aromatica* Salisb. (a) flower and (b) rhizomes (Sharma et al. 2011)

4.3.3. Extraction and Isolation

The air-dried fine powdered rhizomes (2.0 Kg) were subjected to extraction in a Soxhlet apparatus with chloroform for 60 h. The chloroform extraction solvent was removed under reduced pressure to obtain a dark viscous mass. It was adsorbed on silica gel (60–120 mesh) to form slurry, air dried and chromatographed over silica gel (0.75 kg) column packed in petroleum ether.
Before packing in an open column, a preliminary examination of the crude extract mass was done by thin layer chromatography (TLC). This examination gave an idea for elution of the column and the presence of different number of compounds. The TLC plates were prepared manually. The column was eluted successively with PE, mixtures of PE and ethyl acetate (EA) in the ratio of 49:1, 97:3, 19:1, 9:1, 4:1, 3:2, 1:1 and 1:4, v/v (table 1) and finally with chloroform. Various fractions were collected separately and monitored by TLC to check homogeneity. Like fractions are clubbed, concentrated and re-crystallized to afford pure compound(s).

Table 1. Fractionation of the Chloroform - extract

<table>
<thead>
<tr>
<th>Eluent % (Column Polarity)</th>
<th>Fraction No.</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE 100 %</td>
<td>1 -16</td>
<td>Viscous light yellow oil mixture</td>
</tr>
<tr>
<td>PE:EA, 49:1 v/v</td>
<td>17 - 31</td>
<td>Viscous yellow Four spots</td>
</tr>
<tr>
<td></td>
<td>32 - 58</td>
<td>Viscous light yellow Three spots</td>
</tr>
<tr>
<td></td>
<td>59 - 71</td>
<td>Viscous light greenish yellow Single spot (4)</td>
</tr>
<tr>
<td>PE:EA, 97:3 v/v</td>
<td>72 - 89</td>
<td>Viscous light yellow oil Three spots</td>
</tr>
<tr>
<td></td>
<td>90 - 102</td>
<td>Viscous light yellow Two spots</td>
</tr>
<tr>
<td>PE:EA, 19:1 v/v</td>
<td>103 - 118</td>
<td>Viscous light yellow Four spots</td>
</tr>
<tr>
<td></td>
<td>119 - 126</td>
<td>Viscous light yellow Three spots</td>
</tr>
<tr>
<td></td>
<td>127 - 141</td>
<td>Viscous yellow Two spots</td>
</tr>
<tr>
<td>PE:EA, 9:1 v/v</td>
<td>166 - 174</td>
<td>Mixture of four spot (1, 2)</td>
</tr>
<tr>
<td></td>
<td>175 - 187</td>
<td>Mixture of two spots (3)</td>
</tr>
<tr>
<td>PE:EA, 4:1 v/v</td>
<td>188 - 201</td>
<td>Mixture of three spots (5,6)</td>
</tr>
<tr>
<td>PE:EA, 3:2 v/v</td>
<td>202 - 215</td>
<td>Mixture of viscous gummy oils</td>
</tr>
<tr>
<td>PE:EA, 1:1 v/v</td>
<td>216 - 228</td>
<td>Mixture of three spots</td>
</tr>
<tr>
<td>PE:EA, 1:4 v/v</td>
<td>229 - 237</td>
<td>Gummy brown solid mixture</td>
</tr>
</tbody>
</table>
4.3.4. β-Sitosterol-α-D-glucoside (6)

White solid; m.p. 255-260°C(d); IR (ν_max, cm⁻¹) 3360, 2940, 1460, 1369, 1070, 1024; ^1^H NMR (400 MHz, DMSO-d_6) : δ_H 0.63, 0.76, 0.81, 0.88, 0.95, 1.11 (s, 3H each, 6xCH₃), 2.89 (m, 1H, H-3), 3.62 (m, 1H, CH of glucose), 3.10 (m, 1H, CH of glucose), 3.04 (m, 1H, CH of glucose), 4.20 (d, J = 7.5 Hz, 1H, CH of glucose), 4.48 (m, 1H, CH of glucose), 4.92 (m, 2H, CH₂ of glucose), 5.03 (m, 1H, H-1'), 5.30 (m, 1H, H-6); ^13^C NMR (100 MHz, DMSO-d_6) : δ_C 36.7 (C-1), 29.2 (C-2), 76.9 (C-3), 40.0 (C-4), 140.4 (C-5), 121.2 (C-6), 31.3 (C-7), 29.2 (C-8), 49.5 (C-9), 36.2 (C-10), 20.9 (C-11), 39.2 (C-12), 41.8 (C-13), 56.1 (C-14), 25.3 (C-15), 28.6 (C-16), 55.3 (C-17), 12.1 (C-18), 18.9 (C-19), 35.4 (C-20), 19.1 (C-21), 36.1 (C-22), 33.2 (C-23), 45.1 (C-24), 29.2 (C-25), 19.6 (C-26), 20.5 (C-27), 28.6 (C-28), 11.7 (C-29), 100.7 (C-1'), 70.0 (C-2'), 76.9 (C-3'), 73.3 (C-4'), 76.7 (C-5'), 61.0 (C-6'); EIMS : m/z 475.06.
4.3.5. **Semisynthesis of Zederol (7) from Zederone (4):**

A mixture of zederone 4 (0.123 g, 0.5 mmol) in methanol and sodium borohydride (0.105 g, 2.77 mmol) was stirred for 1 hr at 0°C. The reaction mixture was diluted with water and extract with ethyl acetate (2 times). The organic layer was combined and washed with water. A white ppt. was filtered, dried and re-crystallized to afford an alcoholic compound 7. White solid; m.p. 152-154°C; IR (KBr): 3445, 2978, 2934, 2861, 1553, 1447, 1389, 1275, 1140, 934, 882,791 cm⁻¹; ¹H NMR (400 MHz, CDCl₃); δ_H 7.24 (s, 1H, OH), 7.07 (br s, 1H, H-12), 5.30 ( m, 1H, H-1), 3.52 (d, J = 16.0 Hz, 1H, H-9), 3.48 (s, 1H, H-5), 3.30 (d, J = 16.0 Hz, 1H, H-9), 2.97 (s, 1H, H-6), 2.46 (m, 2H, H-2), 2.13 (m, 1H, H-3α), 2.09 (s br, 3H, 11-Me), 1.87 (s, 3H, 10-Me), 1.61 (1.48 (s, 3H, 4-Me), 1.14 (m, 1H, H-3β); MS: m/z 248.92, 226.94, 144.95.

4.3.6. **Transformative reaction of Zederol (7) to Lactone (8):**

Three drops of Ethyl acetate was added to 0.0735 g, 0.5 mmol of 7 in CHCl₃ (4 ml). The reaction solution was stirred for 30 min at room temperature. The CHCl₃ solution was dried and evaporated to afford the lactone 8. IR: 3417.98, 2960.83, 1751.42 cm⁻¹.

4.3.7. **Bromination of Zederone (4)**

A solution of N-bromosuccinimide (N BS)(1.42 g, 0.6 mmol) in dry DCM (2.0 mL) was cooled to 0°C and sequentially added drop wise to a solution of zederone 4 (0.0586 g, 0.4 mmol) in dry DCM (2ml) and dimethyl sulphide (0.0372 g, 0.45 mL, 0.6 mmol) in dry DCM (1mL). The resulting mixture was stirred overnight at RT, diluted with hexane (10 mL) and poured into cold brine.
(30 mL).\textsuperscript{19} The separated aqueous phase was extracted with hexane (3x25 mL) and combined organic solution was washed with brine, dried and concentrated. The residue was purified by column chromatography over silica gel by eluting with PE:EA (99:1, v/v) to afford a brown solid 10. IR: 3165.29, 1697.41, 1373.36, 1193.98, 823.63, 640.39 cm\textsuperscript{-1}.

4.3.8. Bromination of Zederol (7)

Same procedure as above to afford a rearranged product 11. IR: 3392.90, 1710.92, 640.39 cm\textsuperscript{-1}.

4.3.9. Reaction of Zederone (4) with hydrazine\textsuperscript{20,21}

To a stirred, hot (50-60 °C) solution of zederone 4 (0.073 g, 0.5 mmol) in ethanol (3 mL) was added hydrazine hydrate, NH$_2$NH$_2$.H$_2$O (0.024 g, 0.6 mmol) in ethanol (3 mL). The hot mixture was heated at reflux for 24 hr, allowed to cool to 25 °C and filtered and dried to afford 12. White solid; IR (KBr): 2928, 1668, 1443, 1381, 1099, 810 cm\textsuperscript{-1}; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$H 7.27 (s, 1H, H-5), 7.09 (s, 1H, H-12), 5.50 (d, $J$ = 12.0 Hz, 1H, H-12), 3.82 (m, 1H, H-2), 3.74 (m, 2H, H-9), 2.51 (m, H, H-2), 2.27 (m, 2H, H-3), 2.12 (s, 3H, Me-15), 1.60 (s, 3H, Me-13), 1.30 (s, 3H, Me-14).

4.3.10. Reaction of Zederone (4) with phenylhydrazine

To a stirred, hot (50-60 °C) solution of zederone 4 (0.073 g, 0.5 mmol) in ethanol (3 mL) was added phenylhydrazine, C$_6$H$_5$NH-NH$_2$.HCl (0.043 g, 0.3 mmol) in ethanol (3 mL). The hot mixture was heated at reflux for 24 hr, allowed to cool to 25 °C and filtered and dried to afford 13.
Conclusion

*Curcuma aromatica* Salisb. is used as medicine as well as dye. Many sesquiterpenes have been reported from this plant and essential oil components were also reported. The transformative reactions on the isolated sesquiterpenoid, zederone using various reagents are reported. The biological activities of each derivative compound would be analysed. This may lead to the synthesis of new drugs.
References


