EXPERIMENTAL
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Melting point were taken on a Toshniwal apparatus and were uncorrected. IR spectra were recorded on a Perkin Elmer spectrophotometer model 720 in KBr pellet. UV spectra were measured with Cary -14 spectrophotometer using spectral methanol.

$^1$HNMR spectra were taken at 90 MHz in CDCl₃,DMSO-d₆ using tetramethylsilane (TMS) as internal reference. Chemical shift values are recorded in “δ”ppm. Mass spectral analysis were performed on Kratos MS-30 and MS-50 mass spectrometers operating at 70 eV with the evaporation of the sample in the ion source at about 200°C. $^1$H NMR, and MS were recorded with laboratory of Dr.R.Maurya,CDRI,Lucknow. IR and UV spectra were recorded in the Department of Chemistry,Banaras Hindu University. Paper chromatography was done on Whatman No.1 papers and solvent used was BAW (n-BuOH-AcOH-H₂O,4:1:5). Mixtures of K₃[Fe(CN)₆] and FeCl₃ solution was used for visualising phenolic compounds on paper chromatogram. TLC plates were prepared with Si gel G (Centron Research Lab,Bombay) and the spots were visualised by iodine vapour in case of flavonoids.

Anhydrous Na₂SO₄ was normally used for drying organic solvents. Analytical samples were rountiely dried at 80°C over P₂O₅ for 24 hours in vacuo.
The plant material was collected from Mirzapur district, Uttar Pradesh, India and its authenticity was verified by the Department of Botany, Banaras Hindu University, Varanasi. A specimen sample is being preserved in our laboratory.

**ISOLATION PROCEDURE**

The air dried powdered bark of Zizyphus rugosa (3.5kg) was stirred mechanically several times with a mixture of C₆H₆-NH₄OH-MeOH extract was concentrated to one fourth of the volume to remove NH₄OH and MeOH completely. The concentrated C₆H₆ extract was extracted with 7% aqueous citric acid. The aqueous acidic solution was basified with ammonia and extracted with chloroform several times. Crude alkaloids (5.8g) obtained after removing the solvent was chromatographed over silica gel column eluting with solvents of increasing polarity. Each eluants were monitored by TLC. The CHCl₃, CHCl₃-MeOH(9:1),(8:2) (7:3) and (2:1) eluants furnished the Zr-1, Zr-2, Zr-3, Zr-4 and Zr-5 respectively.

The C₆H₆ fraction left after extraction with citric acid was evaporated to dryness. It was chromatographed over silica gel column. Each eluants were monitored by TLC. The CHCl₃-MeOH(50:1), (20:1) and (10:1) eluants furnished respectively the compounds Zr-6, Zr-7 and Zr-8.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield from 3.5 kg of Z.rugosa</th>
<th>Solvent system</th>
<th>$R_f$ value</th>
<th>Staining Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zr-1</td>
<td>15mg</td>
<td>CHCl$_3$-MeOH (9:1)</td>
<td>0.32</td>
<td>Dragendorff’s</td>
</tr>
<tr>
<td>Zr-1</td>
<td>15mg</td>
<td>CHCl$_3$-MeOH (4:1)</td>
<td>0.45</td>
<td>Dragendorff’s</td>
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<tr>
<td>Zr-2</td>
<td>20mg</td>
<td>CHCl$_3$-MeOH (1:1)</td>
<td>0.45</td>
<td>Dragendorff’s</td>
</tr>
<tr>
<td>Zr-3</td>
<td>21mg</td>
<td>CHCl$_3$-Acetone-MeOH (1:1:1.5)</td>
<td>0.75</td>
<td>Dragendorff’s</td>
</tr>
<tr>
<td>Zr-3</td>
<td>21mg</td>
<td>C$_6$H$_6$-EtOAc-MeOH (3:1:0.4)</td>
<td>0.35</td>
<td>Dragendorff’s</td>
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<tr>
<td>Zr-4</td>
<td>18mg</td>
<td>CHCl$_3$-MeOH (9:1)</td>
<td>0.30</td>
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<tr>
<td>Zr-4</td>
<td>18mg</td>
<td>CHCl$_3$-MeOH (4:1)</td>
<td>0.40</td>
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</tr>
<tr>
<td>Zr-5</td>
<td>22mg</td>
<td>C$_6$H$_6$-MeOH (22:2)</td>
<td>0.40</td>
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<tr>
<td>Zr-6</td>
<td>36mg</td>
<td>C$_6$H$_6$-Me$_2$CO (3:2)</td>
<td>0.66</td>
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<tr>
<td>Zr-6</td>
<td>36mg</td>
<td>Pet.ether-EtOAc-AcOH (20:8:1)</td>
<td>0.60</td>
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<tr>
<td>Zr-7</td>
<td>25mg</td>
<td>C₆H₆-EtOAc (1:1)</td>
<td>0.62</td>
<td>Dragendorff’s</td>
</tr>
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</tr>
<tr>
<td>Zr-7</td>
<td>25mg</td>
<td>C₆H₆-Me₂CO-AcOH</td>
<td>0.73</td>
<td>Dragendorff’s</td>
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<td></td>
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<td>(15:10:0.2)</td>
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<tr>
<td>Zr-7</td>
<td>25mg</td>
<td>CHCl₃-MeOH-H₂O</td>
<td>0.85</td>
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<td>(65:35:10)</td>
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<tr>
<td>Zr-8</td>
<td>22mg</td>
<td>CHCl₃-MeOH (1:1)</td>
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<tr>
<td>Zr-8</td>
<td>22mg</td>
<td>CHCl₃-MeOH-H₂O</td>
<td>0.90</td>
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<td>(15:15:6drops)</td>
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</tr>
<tr>
<td>Zr-8</td>
<td>22mg</td>
<td>CHCl₃-MeOH-H₂O</td>
<td>0.65</td>
<td>Dragendorff’s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(65:35:10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Zr-1

Colourless granules (15 mg), mp. 264-265\(^\circ\)C, R\(_f\), value on silica gel G plate:
0.32 (CHCl\(_3\)-MeOH, 9:1), 0.45 (CHCl\(_3\)-MeOH, 4:1), IR \(\nu_{max}(\text{KBr})\) cm\(^{-1}\) 3260 for
\(-\text{NH}\), 3020-2920 for \(-\text{CH}\), 1230 for phenol ether, 1610, 1500 (aromatic), 2890,
2790 for \(-\text{NMe}\), 1652 for \(-\text{C}=\text{C}\), 1624 for \(-\text{C}=\text{C}\), 1620 (sec.
amide). UV\(_{max}^{\text{MeOH}}\) (nm) 205 (strong end absorption).

MS: m/z 534.3220 \([\text{M}]^+\), HRMS calcld. for C\(_{31}\)H\(_{42}\)N\(_4\)O\(_4\) 534.3205,
542, 489, 308, 228, 190, 148, 135, 131, 115, 114. 1288 (C\(_7\)H\(_{16}\)N base peak).

Hydrolysis of Zr-1

Zr-1 was hydrolysed with 6 N HCl in a sealed tube at 120\(^\circ\)C for 20
hours. The hydrolysate was examined by paper chromatography (solvent:
n-BuOH-AcOH-H\(_2\)O, 4:1:5). It showed two ninhydrin positive spots which were
identified as phenylalanine and N,N-dimethylisoleutine, by comparison with
authentic sample.

Zr-2

Colourless granules (20 mg), m.p. 103-104 \(c\)[\(\alpha\)]\(_D\)\(^{25}\)-315 \(^{\circ}\) (c-33 MeOH), R\(_f\),
value on silica gel G-plate: 0.45 (CHCl\(_3\)-MeOH, 1:1), IR \(\nu_{max}(\text{KBr})\) cm\(^{-1}\) 3395 for
\(-\text{NH}\), 2960-2820 (-CH), 2790 (-NCH\(_3\)), 1620 (-C=O), 1220 and 1040 for phenol
ether, 1660 (sec. amide group), 1500 (aromatic). UV(MeOH)\(_{max}(\text{nm})\) 203, 90 MHz
\(^1\) HNMR(CDC\(_3\)) (\(\delta\)): 0.86 (6H, d), 1.22 (3H, d), 2.16 (6H, d), 1.72-3.55 (8H, complex
Hydrolysis of Zr-2

Zr-2 was hydrolysed with 6N HCl in a sealed tube at 120° C for 20 hours. The hydrolysate was checked by paper chromatography (solvent, nBuOH-AcOH-H2O(4:1:5)). It showed two ninhydrin positive spots, which were identified as phenylalanine and N,N-dimethylalanine by comparison with authentic samples.

Zr-3

Colourless amorphous powder (21mg), [α]D20 = -256° (C, 0.1, CHCl3), R value on CHCl3-Acetone-MeOH(1:1:1.5): 0.75 and on C6H6-EtOAc-MeOH(3:1:0.4): 0.35. IR νmax(KBr)cm⁻¹ 3300 for -NH, 3010-2870 for -CH, 2790 for N-Methyl, 1630 for styryl double bond, 1600 and 1500 for aromatic, 1214 and 1025 for phenol ether, 1690 for sec.amido group. UV(MeOH) λmax(nm) 235 and shoulders at 250 and 280nm. 90 MHz 1 H NMR(CDCl3)(δ): 0.6-1.0(18H, m), 1.5-1.70(3H, complex pattern), 2.3 (6H, s), 2.6-3.70(8H, complex pattern), 4.0-4.9(6H, complex pattern) 5.50(1H, complex pattern) 6.25(1H, d) 6.3-7.3(8H, complex pattern).
Hydrolysis of Zr-3

Zr-3 was hydrolysed with 6N HCl in a sealed tube at 120°C for 20 hours. The hydrolysate was examined by paper chromatography (solvent, n-BuOH-AcOH-H₂O, 4:1:5). It showed three ninhydrin spots, which were identified as leucine, isoleucine and N,N-dimethylisoleucine by comparison with authentic sample.

Zr-4

Colourless amorphous powder (18mg). Rf value on silica gel G-plate: 0.30 (CHCl₃-MeOH, 4:1). IR vₘₐₓ(KBr) cm⁻¹: 3350-3200 (-NH), 2790(-NCH₃), 1680, 1640 (sec. amido group), 1620(-C=C-), 1220 (phenolether). UV(MeOH) λₘₐₓ(nm): 210. MS: m/z 665.3587, [M⁺], HRMS calcld. for : CₓH₁₇N₅O₅ 665.3576, 662, 622, 574, 518, 378, 376, 308, 243, 229, 215, 209, 203, 186, 148.1128 (C₁₀H₁₄N, base peak).

Hydrolysis of Zr-4

Zr-4 was hydrolysed with 6N HCl in a sealed tube at 120°C for 20 hours. The hydrolysate was checked by paper chromatography (solvent: n-BuOH-AcOH-H₂O, 4:1:5). It showed three ninhydrin positive spots in the hydrolysate
which were identified as isoleucine, phenylalanine and N,N-dimethylphenylalanine by comparison with authentic samples.

**Zr-5**

Colourless granules (22mg) m.p.-237-239°C, [α]D20 - 43° (C,0.06,MeOH), R value :0.4 (benzene:methanol,22.2). IR Vmax(KBr) cm⁻¹ :3270(-NH),2980-2830(-CH),2785(-NCH₃),1675,1625 (sec.amido group), 1610(-C=C)-, 1230 and 980 (phenol ether). UV(MeOH)λmax(nm) 223 and shoulers at 290, 281 and 273. H NMR (CDCl₃-DMSO)(δ) : 0.51-0.72 (6H, d), 0.67-1.18 (4H, complex pattern), 0.97-1.22 (6H, d), 2.30 (6H, s), 3.10-3.45 (3H, complex pattern), 3.90 (1H, d, d), 4.47 (1H, d), 4.97 (1H, d), 6.0-7.9 (14H, complex pattern), 9.31 (1H, s). MS: m/z 573.3284[M⁺], HRMS calcld for: C₃₃H₄₅N₅O₄ 573.3286, 443, 387, 303, 274, 195, 190, 187-1338 (C₁₂H₁₅N₂, base peak), 182, 167, 135, 130, 97, 86.

**Hydrolysis of Zr-5**

Zr-5 was hydrolysed with 6N HCl in a sealed tube at 120°C for 20 hours. The hydrolysate was checked by paper chromatography. It showed two ninhydrin positive spots in the hydrolysate which were identified as 3-hydroxyleucine and leucine by comparison with authentic samples.
Zr-6

Yellow granules (36 mg) m.p. 226-228°C, \( R_f \) value with \( C_6H_5- 
Me_2CO(3:2):0.66 \) and pet.ether-EtOAc-AcOH (20:8:1):0.60. IR \( \nu_{max}(\text{KBr}) \) cm\(^{-1}\), 3300-3600 and 1665 for strong phenolic-OH and chelated carbonyl groups respectively. UV \( \lambda_{max}(\text{MeOH}): 250\text{sh}, 268, 300, 320, 365 \) (log \( e \) 4.16, 4.22, 3.71, 3.95, 4.30); 90 MHz \(^1H \) NMR (DMSO-d\(_6\)) (\( \delta \)): 3.85 (3H, s, -OMe), 6.34 (1H, d, \( J 2.2\text{Hz}, H-6 \)), 6.72 (1H, d, \( J 2.2\text{Hz}, H-8 \)), 6.93 (2H, d, \( J 9\text{Hz}, H-3'), H-5' \)), 8.10 (2H, d, \( J 9\text{Hz}, H-2', H-6' \)) 9.51 (1H, s, -OH, exchangeable with D\(_2\)O), 10.15 (1H, s, -OH, exchangeable with D\(_2\)O); MS: \( m/z \) 300.0632[M\(^+\)], HRMS calcld. for C\(_{16}H_{12}O_6\), 299, 272, 271, 258, 257, 229, 213, 181, 152, 148, 124, 121, 119, 69.

Methylation of Zr-6

Zr-6 on methylation with CH\(_2\)N\(_2\) gave trimethylderivative, mp 148-159°C; C\(_{19}H_{18}O_6\)(m/z 342,1100[M\(^+\)]) which showed three more methoxyl groups in \(^1H \) NMR at \( \delta \) 3.88, 4.15 and 4.24.

Zr-7

Yellow crystalline solid, mp 320-322°C; \( R_f \) value of 0.62 on \( C_6H_6- 
EtOAc(1:1), 0.73 \) on \( C_6H_6-Me_2CO-AcOH(15:10:0.2), 0.85 \) on CHCl\(_3\)-MeOH-H\(_2\)O(65:35:10). IR \( \nu_{max}(\text{KBr}): 3245-3450 \) (-OH), 1640 (conjugated carbonyl), 1595 cm\(^{-1}\) (aromatic nucleus); UV(MeOH) \( \lambda_{max}(\text{nm}): 250, 267, 290 \) sh, 350 (log
e4.25, 4.22, 4.00, 4.33; 90 MHz $^1$H NMR (DMSO-d$_6$) (δ): 6.26, 6.64, (1H, d, each, J 2 Hz, H-6, H-8), 6.82 (1H, s, H-3), 6.95 (1H, d, J 9.5 Hz, H-5), 7.50 (1H, d, J 9.5, 2.0 Hz, H-6'), 7.52 (1H, d, J 2 Hz, H-2'), 12.95 (1H, s, -OH, exchanged with D$_2$O); Ms: m/z 286.0478 [M$^+$], calcd. for C$_{15}$H$_{10}$O$_6$+$^{10}$O, 258, 257, 229, 153, 152, 134, 129, 124, 69

**METHYLATION OF Zr-7**

On methylation with Ac$_2$O and triethylamine, Zr-7 furnished an acetate, m.p. 225-228°C; C$_{23}$H$_{18}$O$_{10}$ (HRMS: m/z 454.0900 [M$^+$]) which exhibited four acetate groups in $^1$H NMR at δ 2.32 (3H, s, 1xOAc), 2.33 (6H, s, 2xOAc), 2.43 (3H, s, 1xOAc).

**Zr-8**

Yellow granules, mp 184-186°C; Rf value of 0.55 on chloroform-methanol (1:1), 0.90 on chloroform-methanol-water (15:15:6 drops), 0.65 on chloroform-methanol-water (65:35:10). IR $\nu_{max}$ (KBr): 3200-3390 br, 1642, 1594 cm$^{-1}$; 90 MHz $^1$H NMR (DMSO-d$_6$) (δ): 3.30-4.00 (6H, m, glucosyl protons), 5.00 (1H, br s, anomeric-H), 6.45, 6.82 (1H, d, each, J 2 Hz, H-6, H-8), 6.76 (1H, s, H-3), 6.94 (1H, d, J 9 Hz, H-5'), 7.50 (1H, d, d, J 2 Hz, H-2'), 7.55 (1H, d, J 2 Hz, H-2'), 12.95 (1H, br s, -OH); UV(MeOH)$\lambda_{max}$(nm): 254, 265, 294sh, 345 (logε .56, 3.52, 3.26, 3.50).
Hydrolysis of Zr-8

Zr-8 was hydrolysed with 6%aq.HCl in MeOH for 4 hours. The hydrolysate was checked by paper chromatography. It showed yellow granules which was identified as luteolin by IR, UV, $^1$H NMR, MS and the sugar in the hydrolysate was identified as glucose by comparison with authentic sample.