Isolation and Characterization of Urosolic Acid from Marketed “Uriflux” Polyherbal Diuretic Syrup

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Abstract: Isolation of polyherbal formulation by chromatographic technique is vital keeping in mind the end goal to find out survey the nature of the medications, taking into account the concentration of active principles. "Uriflux" syrup. A Polyherbal syrup holding 5 herbs materials in manifestation of water concentrate, which acting with fundamental diuretic standards, which are Mimusops elengi, Santalum album, Crateva nurvala, Tribulus terrestris, Andropogon muricatus. Active diuretic constituent Urosolic acid isolated from syrup, working for urinary problem and its characterization is done. Structural elucidation done by IR, NMR AND GC-MS for exact identification.

Keywords: URIFLUX syrup, urosolic acid, , Diuretic, structural elucidation

Introduction[1,2,6,7]:

Nowadays there are Ayurvedic products in great demand in the all countries of the world for primary health care because of their a lot of biological activities, higher safety margins and lesser costs. Public and government likeness towards herbal medicines is growing fast due to increased incidence of the adverse drug reactions and economics of the modern system of medicine. Uriflux is polyherbal diuretic syrup containing five crude drug decoction containing chemical constituents wich acting on different urinary problems. The present study is on isolation of urosolic acid from marketed uriflux syrup. Ursolic acid (3β-hydroxyurs-12-en-28-oic acid) is a pentacyclic triterpenoid. There are different activities reported for urosolic acid like analgesic, anti-tumor, anti-diabetic, anti-oxidant, COX-2 inhibitor, anti- inflammatory, hepatoprotective, anti-HIV anticancer, antibacterial, anti-inflammatory etc. It is present in moolsari phool. The marketed Uriflux syrup consist following crude drug extract

1. Moolsari Phool (Mimusops elengi, Family- Sapotaceae) - 7gram
2. Gokharu (Tribulus terrestris, Family- Zygophylaceae) - 5gram
3. Varuna chhal (Crateva nurvala, Family- Capparaceae) - 5gram
4. Wala mool (Andropogon muricatus, Family-Poaceae) - 3gram
5. Chandan (Santalum album, Family- Santalaceae) - 1gram

Uriflux polyherbal syrup consist different chemical constituent like lupeol, varunol, santalol, urosolic acid etc. But we focused on urosolic acid which acting as antibacterial is isolated.

Isolation is done by Column chromatography using silica gel G,60-120 mesh size and mobile phase optimized-chloroform : methanol (8:2) proportion. The chemical tests for steroid are performed of urosolic acid which is important for urinary problems.
Experimental[7-10]:

Marketed polyherbal “URIFLUX” syrup procured from Bewell Pharmaceuticals Kagal MIDC, Kolhapur, Maharashtra, India. All the chemicals and reagents used were of analytical grade IR spectra- JASCO FTIR-410, NMR-BRUKER EVANS-300MHZ, GCMS- SHIMADZU JAPAN QP 201 Glass column BOROSIL MUMBAI. Such types of models are used.

Isolation and Purification Of Compound-

Column chromatography using silica gel G, 60-120 mesh size and mobile phase optimized-chloroform : methanol (8:2) proportion for gradient elution gave a colourless solid.

This was further purified by silica gel chromatography and again eluted with chloroform: methanol (8:2) to obtain ursolic acid.

Structural Elucidation of Urosolic Acid[3,4,5]:

a) IR: IR of isolated Urosolic acid is done by, JASCO FTIR-410.

b) NMR: 1H NMR of isolated urosolic acid is done by using CDCl3, from Shivaji university Kolhapur.

c) GCMS: It is done from Shivaji university Kolhapur.

Result and Discussion:

1) IR:

![Infrared spectroscopy](image-url)

Figure no:-1) Infrared spectroscopy

Table no:-1) Infrared spectroscopy

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Wave No.(cm⁻¹)</th>
<th>Description (Functional group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>3565.4</td>
<td>O-H bond vibrations</td>
</tr>
<tr>
<td>15</td>
<td>2924.52</td>
<td>O-H bond vibrations of COOH</td>
</tr>
<tr>
<td>18</td>
<td>1732.73</td>
<td>C = O vibration</td>
</tr>
<tr>
<td>22</td>
<td>1652.70</td>
<td>C=C vibration (aromatic)</td>
</tr>
<tr>
<td>27</td>
<td>1473.35</td>
<td>bending vibration of C-H</td>
</tr>
<tr>
<td>30</td>
<td>798.39</td>
<td>Out of plane C – H vibration</td>
</tr>
</tbody>
</table>

2) NMR: Proton NMR of Urosolic acid was done by BRUKER EVANS-300MHZ. The spectra displayed different δ values. 7.28 singlet, 3.6 singlet, 1.591 singlet, 1-1.5 multiplate.
Figure no 2 :- Nuclear magnetic spetroscopy of urosolic acid

Table no. 2: proton NMR –urosolic acid

<table>
<thead>
<tr>
<th>δ(300MHz,CDCl₃)</th>
<th>Proton number</th>
</tr>
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<tbody>
<tr>
<td>7.281</td>
<td>21H, singlet</td>
</tr>
<tr>
<td>3.6</td>
<td>2H, singlet</td>
</tr>
<tr>
<td>1.59</td>
<td>18H, singlet</td>
</tr>
<tr>
<td>1.1-1.5</td>
<td>7H, multiplate</td>
</tr>
</tbody>
</table>

From result it concludes that different bands obtained confirms structure of Urosolic acid.

**GCMS:** GC spectrum shows peaks at different retention times. The mass spectrum of the peak at 24.750 RT was analyzed. Molecular ion peak at [M]+ m/z 456 that correspond to molecular formula C₃₀H₄₈O₃. The ion peak at m/z 414 depicts the loss of – COOH group i.e. M- 45, and gain of three hydrogen i.e M+3.
Discussion:

The different bands obtained in IR ,retention time 24 , mass peak of GCMS 414 and chemical shift in NMR are matching and nearest to the standard urosolic acid literature. So result of experimental work shows that isolated sample is Urosolic acid.

Conclusion:

From the data of structural elucidation of Urosolic acid it is proved that isolated compound from marketed herbal diuretic syrup is Urosolic acid which is important for antibacterial activity in Uriflux polyherbal syrup.

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References

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