

## Isolation and Characterization of Urosolic Acid from Marketed "Uriflux" Polyherbal Diuretic Syrup

Patil Pallavi N\*, Nitave Sachin A.

J.J. Magdum Trust's, Anil Alias Pintu Magdum Memorial College of Pharmacy,  
Dharangutti, Tal-Shirol, dist-Kolhapur, State-Maharashtra, India.

\*Corres.author: reachpallu58@gmail.com, Mobile no- 8600399659, 9730086658

**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<sup>[1,2,6,7]</sup>:

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 wick acting on different urinary problems. The present study is on isolation of urosolic acid from marketed uriflux syrup. Ursolic acid (3 $\beta$ -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 ( <i>Mimusops elengi</i> , Family- Sapotaceae)  | -7gram |
| 2. | Gokharu ( <i>Tribulus terrestris</i> , Family- Zygophyllaceae) | -5gram |
| 3. | Varuna chhal ( <i>Crateva nurvala</i> , Family- Capparaceae)   | -5gram |
| 4. | Wala mool ( <i>Andropogon muricatus</i> , Family-Poaceae)      | -3gram |
| 5. | Chandan ( <i>Santalum album</i> , 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<sup>[7-10]</sup>:**

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<sup>[3,4,5,]</sup>:**

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

b) **NMR:** <sup>1</sup>H NMR of isolated urosolic acid is done by using CDCl<sub>3</sub>, from Shivaji university Kolhapur.

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

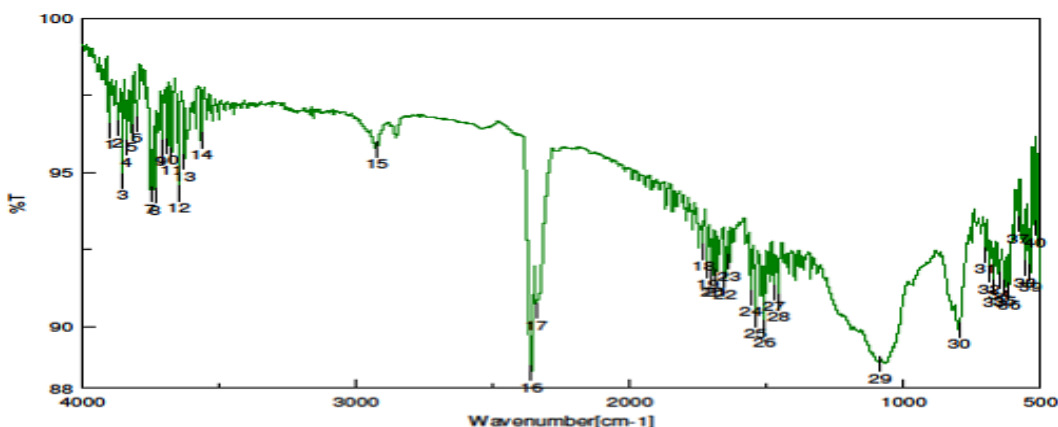
**Result and Discussion:****1) IR:**

Figure no-:1) Infrared spectroscopy

Table no-:1) Infrared spectroscopy

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

2) **NMR:** Proton NMR of Urosolic acid was done by BRUKER EVANS-300MHZ. The spectra displayed different  $\delta$  values. 7.28 singlet, 3.6 singlet, 1.591 singlet, 1-1.5 multiplate.

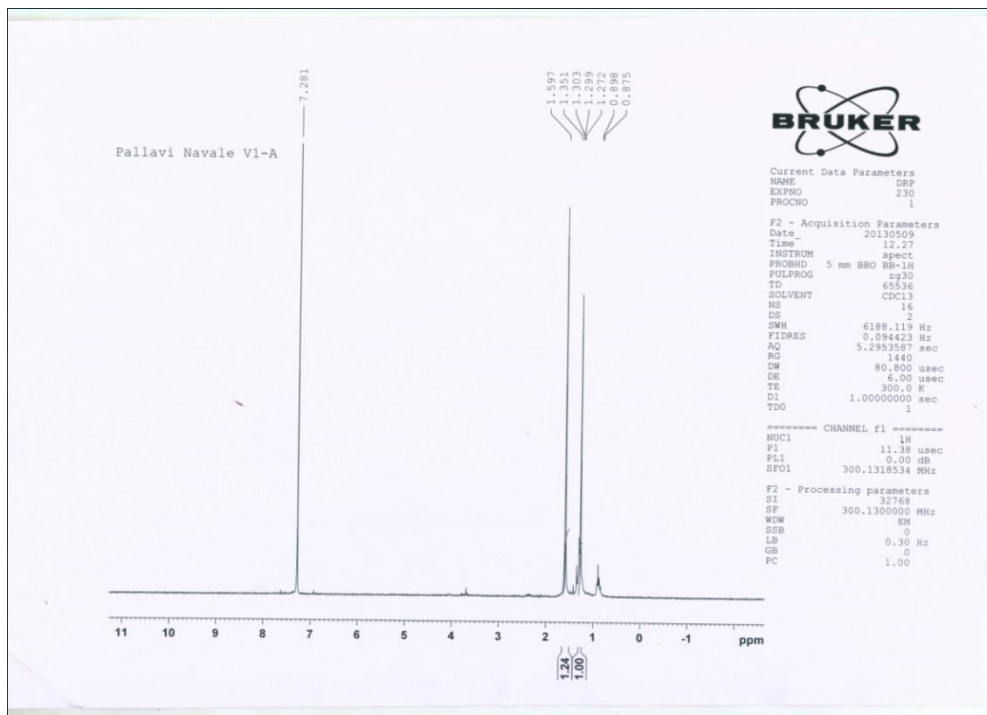
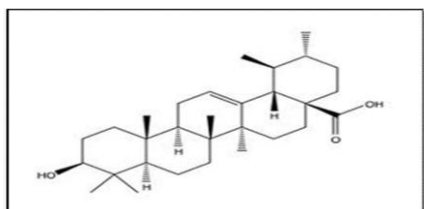


Figure no 2 -: Nuclear magnetic spectroscopy of urosolic acid

Table no. 2-: proton NMR –urosolic acid

$\delta(300\text{MHz}, \text{CDCl}_3)$	Proton number
7.281	21H, singlet
3.6	2H, singlet
1.59	18H, singlet
1.1-1.5	7H, multiplate



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  $\text{C}_{30}\text{H}_{48}\text{O}_3$  The ion peak at  $m/z$  414 depicts the loss of  $-\text{COOH}$  group i.e.  $M-45$ , and gain of three hydrogen i.e  $M+3$ .

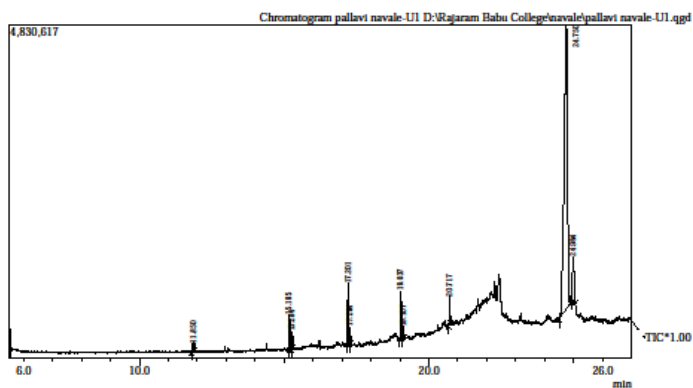


Figure no-:3) Gas chromatography

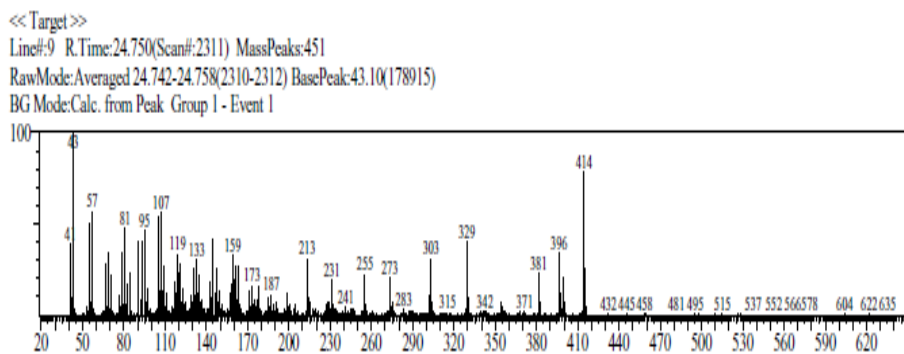


Figure no-: 4) Mass spectra from GCMS

### 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..

### Acknowledgement:

Author thankful to Dr. C.S.Magdum , Principal of RCP Kasegaon, and my family, my friends, for their support.

### References

1. Akhtar N, Ali M\* and Alam MS, Pentacyclic triterpenes from the stem bark of *Mimusops elengi*, Acta poloniae Pharmaceutica in Drug Research, 2009; 66( 5): 549-552.
2. Gami B, Pathak B, Parabia M, Ethnobotanical, phytochemical and pharmacological review of *Mimusops elengi* Linn, Asian Pacific Journal of Tropical Biomedicine, 2012; 1691(12):741-748.
3. S: Sharma YR, Elementary organic spectroscopy principle and chemical applications, S Chand publication, multicolour edition, 69-235.
4. Kemp W Organic spectroscopy, Palgrave publication, 3<sup>rd</sup> edition; 2011:103,249.
5. Introduction to Fourier Transform Infrared Spectrometry, Thermo Nicolet publication, 2-3.
6. World Health Organization guidelines for assessing quality of herbal medicines with reference to contaminants and residues, WHO Library Cataloguing Publication;2007:1-69.
7. Krishna KL Agrawal SS, Diuretic activity of Sufoof-e-suzak qawi an unani polyherbomineral formulation, Iranian Journal of Pharmacology & Therapeutics, 2006;5(1): 167-169.
8. Kakrani PH, Kakrani BH, Kakrani HN, Isolation of stigmasterol and urosolic acid from methanolic extract of aerial parts of *Alysicarpus Monilifel* (DC.)(Papilionaceae), International Journal of Pharmaceutical Sciences, 2012;3 (3):2185-2191.
9. Leea TH , Juangb SH, Hsua FL and Wua CY, Triterpene Acids from the Leaves of *Planchonella duclitan* (Blanco) Bakhuzian, Journal of the Chinese Chemical Society;2005, 52(1):1275-1280.
10. Chinna E M, Elumalai A, Habibur R, Isolation of phytochemical constituents from stem barks of *Madhuca longifolia*, International Research Journal of Pharmaceutical and Applied Sciences, 2011; 1(1):43-60.