



Extraction, Isolation and Characterization of Phytoconstituents in *Urginea wightii*

M. Smitha Raj^{1*} and M. N. Shiva Kameshwari¹

¹*Department of Botany, Bangalore University, Jnana Bharathi Campus, Bangalore-560 056, India.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The *Urginea wightii* is a well-established squill. The therapeutic activity is determined by the presence of pharmacological component present in the crude extract of *Urginea wightii*. To screen these active herbal components in a single plant material it requires a sophisticated, sensitive and a reliable analytical technique. As a preliminary screening study infrared spectrum provided information related to active functional groups of bulbous extract. This encouraging result evoked us for further accurate detailing of chemical components by advanced analytical techniques like liquid chromatography mass spectroscopy (LC-MS) and Nuclear magnetic resonance (NMR). The identified chemical constituents with possible molecular structures of pharmacological activity by LC-MS was found to be stigmaterol, hexadecanoic acid methyl ester and 1,3,7,11,15-tetramethyl-2-hexadecenol. Similarly, when ¹H NMR was performed an interesting molecule (2,3-dihydro-dihydroxy-6-methyl-4H-pyran-4-one) having potential capability in destroying free radicals (antioxidant) was obtained. The comprehensive and qualitative characterization of these bioactive compounds present in *Urginea wightii* can be a useful in treating vast disease conditions. This investigation paves the way to explore researchers for further use of *Urginea wightii* as sources of medicinally interesting compound.

*Corresponding author: E-mail: smitharaj464@gmail.com;

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1. INTRODUCTION

Urginea is a class of polyphytic genus belongs to family hyacinthaceae mainly concentrated in the geographical regions of Africa (Mediterranean region) and India (Plains of south India). Nearly about 100 different species of *Urginea* are being identified in various parts of the globe. Some of the literatures notify the major contribution of *Urginea* species in treating cancerous cells present in female reproductive track (ovaries), breast, geriatric male reproductive track (prostate) and intestine [1,2]. *Urginea wightii* is one variant containing active ingredients of life saving medicinal qualities [3]. This pear-shaped squill has variety of active components structured in the form of steroids, esters and heterocyclic compounds [4]. A diversified health benefits in the form of anti-cancer, anti-inflammatory, anti-cholesteremic, anti-microbial, pesticidal and anti-HIV can be obtained with the use of this polyphytic extracts. This encourages the researchers and clinicians to explore synergistic action of current bio-analytical techniques and folk medicine to overcome modern world's life threatening diseases. Moreover, India is a country having a strong belief in traditional system of treatment (Ayurveda, Sidda, Unani and Homeopathy). The present work aims in extraction and isolation of active herbal constituents of *Urginea wightii* in benzene and chloroform (alone and in combination) and analyzing the same using modern analytical tools. Initially the presence of different functional groups in the isolated material was detected using Fourier Transform Infra red (FT-IR) followed by qualitative analysis. The compounds present in its pure form were determined by running the Liquid chromatography-mass spectroscopy (LC-MS) and Nuclear magnetic resonance (NMR) [5]. All the phytochemical analytical investigation confirmed the presence of multiple Bufadienolide components such as stigmasterol, methyl ester of hexadecanoic acid, (Stigmasta-4,6,22-triene-3-yl) acetate, 4-(3,5-dimethyl-H-pyrazol-1-yl) 4-oxobutanol etc. This research method can be taken for future process that may include screening and biological testing of *Urginea wightii* for its clinical importance.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Collections of the bulbs were made by regular field trips. Identification of the collected plants,

the fresh and mature bulb of *Urginea wightii* were collected from Yediyur (Karnataka). The collected bulbs were washed thoroughly with water, dried under shade and cut in to small pieces and finally ground (mixer) to fine powder for extraction of crude extract.

2.2 Preparation of Extraction

2.2.1 Cold extraction method

Weigh the dried powder (1000 gm) and added into different conical flask with methanol solvent (10000 ml) and allow keeping at room temperature for thirty-minute shaking after each twenty-four hours for seven days. Finally filter the extract using whatman filter paper under vacuum and dry it at room temperature in watch glass dish (100 gm) [6].

2.3 Isolation

2.3.1 Column chromatography

The methanolic extract of *Urginea wightii* was subjected to silica gel column chromatography (Column height 63 cm; width 3 cm; Silica gel: Acme's 100-200 mesh) using a gradient solvent system of benzene with increasing amounts of benzene-chloroform, chloroform, ethyl acetate-chloroform, ethyl acetate, ethyl acetate-methanol and finally with methanol. Elutants collected from column chromatography were concentrated using rotary vacuum evaporator [7].

2.4 Fourier Transform Infrared Spectroscopy (FT- IR)

Urginea wightii extract was subjected to FT-IR analysis for identification of functional groups and interpreting the same. All the solvent system was purchase from S.D fine chemicals. Fourier Transform Infra-red (FT- IR) Spectroscopic test was performed to study different functional groups present in the *Urginea wightii* species. The extracts of *Urginea wightii* was qualitatively analyzed by squeezing between Potassium Bromide (KBr) windows that has a thickness of 0.01 mm inside the cell [8]. As a part of solvent selection criteria, Non-polar solvents like benzene and chloroform in different ratios were considered for detecting the key functional groups present in *Urginea wightii*. The above mixture of solvents in different ratio were selected based on its peak detecting capability

and absorption potential. After treating the extract in different solvent systems and subjecting to KBr milling technique the thin films was placed in the sample holder of IR Spectrometer (Perkin Elmer Spectrum, Version 1003.07) and scanned from 4000-400 cm^{-1} for presence of functional groups. This mid infrared region was selected in the present investigation as it can cover the entire range of vibrational and rotational transitions. Thus, obtained graphs from spectral signals were interpreted for ease of identifying the functional groups in *Urginea wightii* [9].

2.5 Liquid Chromatography- Mass Spectroscopy (LC-MS)

LC-MS is an advanced analytical tool used to segregate complex mixtures present in the both herbal and synthetic compounds. In this particular study column chromatographic samples of *Urginea wightii* was subjected to qualitative analysis for presence of active component by LC-MS using Agilent instrument assembly (model: Agilent 6410A Triple Quad series make: Agilent technologies, USA). The concentration of mobile phase comprises of 0.1% formic acid aqueous solution (A) and methanol (B) mixed in a ratio of 20:80 (A:B). A flow rate of 0.5 ml/min was maintained following isocratic elution at the temperature of $35 \pm 0.5^\circ\text{C}$. The spectroscopic scan was performed in both positive and negative mode (MS_2) using an atomic mass unit ranging from 100-1000AMU [10].

2.6 Nuclear Magnetic Resonance (NMR) (^1H NMR and ^{13}C NMR)

The methanolic extract of *Urginea wightii* was exposed to NMR instrument (MAKE AND MODEL). The sample was mounted on a NMR probe surrounded by the super conducting magnet. Furthermore, the sample was brought to the excited state in the presence of Dimethyl Sulfoxide (DMSO). A radio frequency wave of 60-1000MHz pulse was maintained by RF coils inside the NMR probe. A NMR spectral response of free induction decay (FID) is obtained consisting of 16 repetitions. Finally, these FID signals are detected by the probe that is sent to the host computer to convert time domain into the frequency domain through process Fourier transformation (FT). All the chemical structures obtained from the LC-MS and NMR studies were designed and drawn using ChemsSketch (demo version 11.0) software [11].

3. RESULTS AND DISCUSSION

3.1 Fourier Transform Infrared Spectroscopy (FT- IR)

As a potential tool, Infrared spectroscopy gives a structural elucidation, chemical interactions and functional group present in the active molecule. In Figs.1-4 the IR spectrum of *Urginea wightii* was found to show distinct peaks when different solvent system was used [12,13]. The results of FT- IR obtained are reported in the Table 1.

Table 1. The results of FT- IR

Sl no	Solvent system	Type of bond	Peaks (cm^{-1})
01	Benzene (100%)	C-H stretching	2924
02		C=C stretching	1733
03		C-H bend	1463
01	Benzene: Chloroform (90:10)	C-H stretching	2924
02		C-H stretching	2852
03		C=C stretching	1713
04		C-H bend	1465
01	Benzene: Chloroform (60:40)	C-H stretching	2924
02		C-H stretching	2853
03		C=C stretching	1714
04		C-H bend	1464
05		C-H bend	1460
06		C-C stretching	1187
07		CH ₂ rocking	721
01	Chloroform (100%)	O-H stretching	3369
02		C-H stretching	2852
03		C-H stretching	2924
04		C=C stretching	1761

Benzene (100%): The benzene extract of *Urginea wightii* exhibited a characteristic peak at 2924cm^{-1} (C-H stretching), an alkene stretching (C=C) was observed at 1733cm^{-1} and a C-H bending was captured at 1463cm^{-1} respectively.

Chloroform (100%): *Urginea wightii* extract subjected to chloroform liquid IR analysis showed the presence of two distinct absorption peak at 2924 and 2853cm^{-1} for C-H stretching. A stretching of alkene group (C=C) at 1713cm^{-1} and C-H bend at 1465cm^{-1} also appeared during the study.

Benzene: Chloroform (90:10 and 60:40): The use of binary solvent system (Benzene: Chloroform) portrayed a distinctive peak of alkane (C-H stretching) at 2924cm^{-1} and 2852, an absorption band at C=C stretching was seen at 1713cm^{-1} and C-H bend at 1465cm^{-1} . Even after varying the ratio of benzene: chloroform (60:40) the absorption bands very matching to each other. But there were two extra bands of C-H bend (1460cm^{-1}) and CH₂ rocking (721cm^{-1}) was produced.

3.2 Liquid Chromatography- Mass Spectroscopy (LC-MS)

The Liquid Chromatography Mass Spectroscopy (LC-MS) of different solvent extract of *Urginea wightii* shown in Figs. 5a-8b. Based on the mass spectroscopic analysis, when benzene (100%) alone was used for extraction and isolation of chemical compound from *Urginea wightii*. A

recognizable signal gave positive response for two components namely a phytosterol and a fatty acid metabolite having molecular formula as $\text{C}_{29}\text{H}_{48}\text{O}$ and $\text{C}_{17}\text{H}_{34}\text{O}_2$ respectively. The peaks obtained from this test confirmed structural resemblance to Stigmasterol ($\sim 413\text{g/mol}$) and Methyl ester of hexadecanoic acid ($\sim 270\text{g/mol}$) as possible analytes present in the extract [14]. Furthermore, combination of solvents benzene: chloroform (90:10) used in extraction of active component in *Urginea wightii* subjected to LC-MS was able to yield chemical constituent namely 1,3,7,11,15-tetramethyl-2-hexadecenol having equal pharmacological importance comparable to stigmasterol and methyl ester of hexadecanoic acid. The extracted constituents from *Urginea wightii* in benzene: chloroform (60:40) was able to produce peaks showing chemical entities of (Stigmasta-4,6,22-triene-3-yl) acetate and 4-(3,5-dimethyl-H-pyrazol-1-yl) 4-oxobutanol. Moreover, the chloroform (100%) extracted sample were able to produce mass spectroscopic peak that corresponds to 4-H-pyran-4-one. None of the literature survey depicts the importance and therapeutic activity of these two chemical constituents. The structures with its chemical name are depicted as follows [15].

The LC-MS results in the present work indicated the presence of mixture of steroidal and methyl ester fatty acid component. These identified components play a vital role in the field of medicines. As a herb, *Urginea wightii* bulb is able to produce these molecules (Stigmasterol) of medicinal importance in fighting certain type of

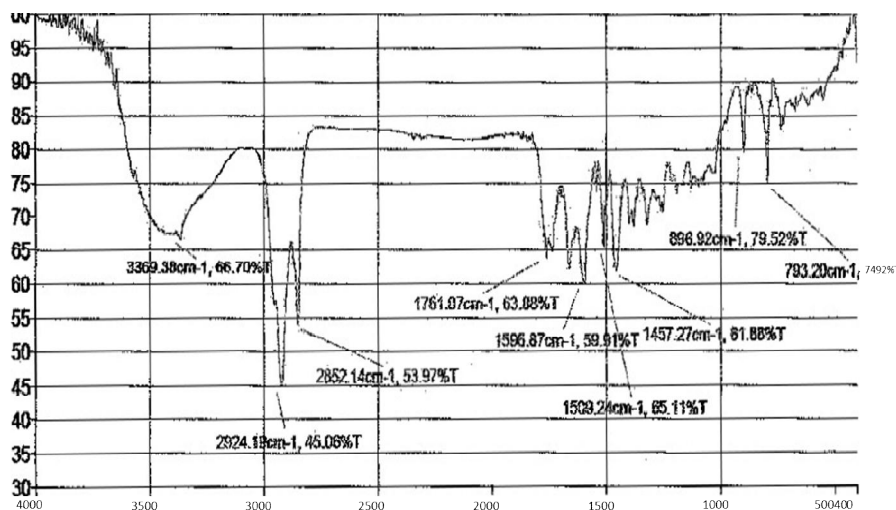


Fig. 1. FT- IR analysis of isolated compound of Benzene (100%) of *Urginea wightii*

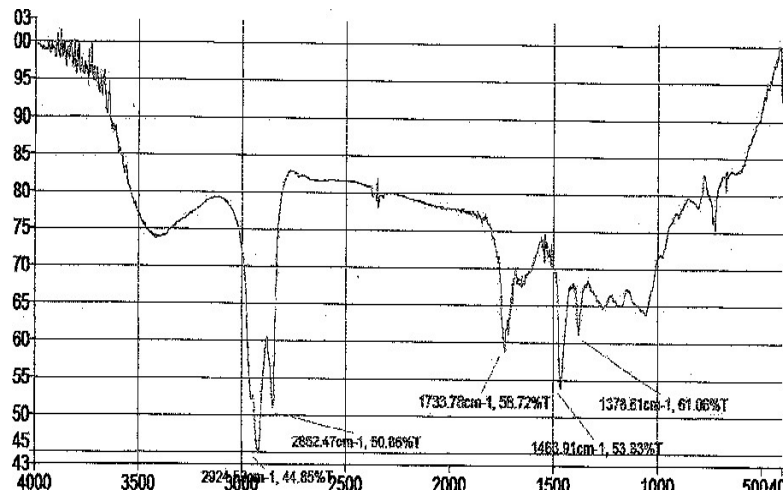


Fig. 2. FT- IR analysis of isolated compound of Benzene: Chloroform (90:10) of *Urginea wightii*

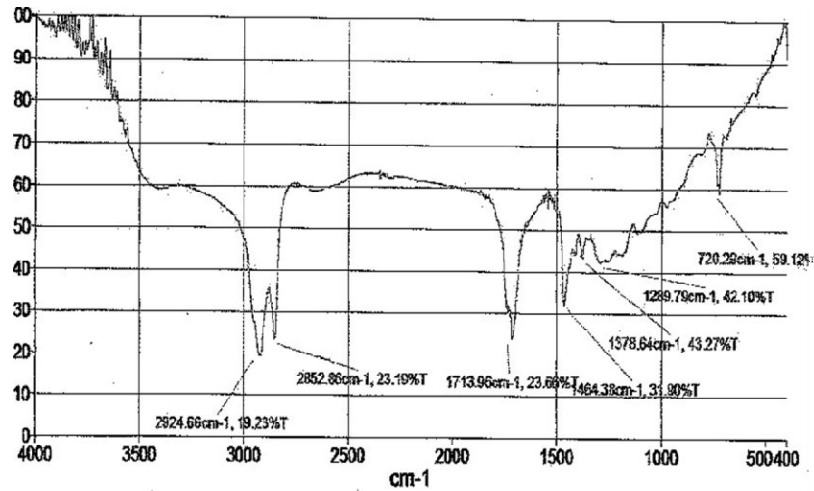


Fig. 3. FT- IR analysis of isolated compound of Benzene: Chloroform (60:40) of *Urginea wightii*

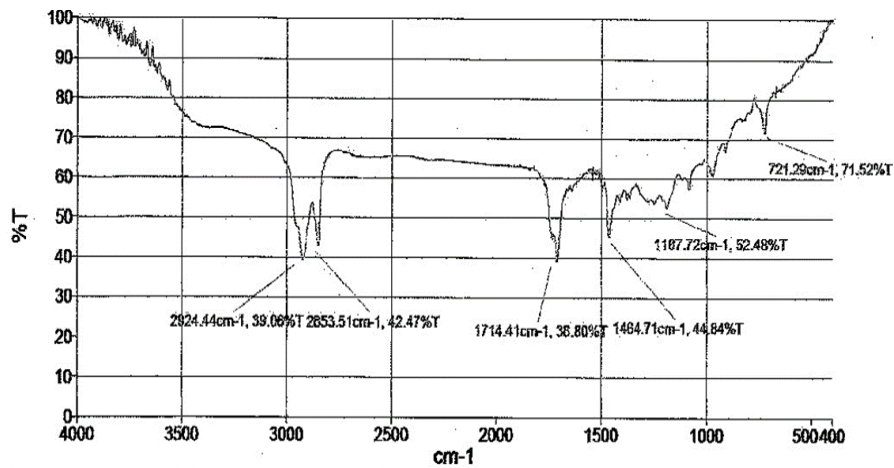


Fig. 4. FT- IR analysis isolated compound of Chloroform (100%) of *Urginea wightii*

cancerous tumors affected to ovaries, prostate, breast and colon. Many research finding indicates that stigmasterol is having its action in regulating the blood sugar levels especially in hypoglycemic patients. Excessive production of thyroxine hormone from the thyroid gland results in a condition called hyper-thyroidism [16]. Stigmasterol, a bufadienolide derivative was found to have its beneficial effect in inhibiting the production of this hormone from the thyroid cells. As the molecule is steroidal in nature it hinders the uptake and absorption of saturated fatty acid. Saturated fatty acid is the main source for low density lipoprotein (LDL) in the human body that leads to cardiac arrest and failure. Stigmasterol has the ability to attack and limit serum cholesterol that in turn lowers the LDL level. Similarly, hexadecanoic acid methyl ester is found be useful in treating high levels of cholesterol in the blood which is an indicative of hypercholesterolemia. Stigmasterol can undergo series of chemical reaction to inhibit reverse transcriptase enzyme that is considered to be a key catalytic component in HIV cases. Literature survey reveals that hexadecanoic acid possess antioxidant property that helps in protecting the healthy cells from damage due to free radicals formation. Plants growth and development is hindered in the presence of microscopic parasite called as nematodes. Hexadecanoic acid and its methyl ester acts as a broad spectrum nematocide much useful as plant parasite. Studies suggest that hexadecanoic acid and its methyl ester can be employed as flavouring agent in food and confectioneries. Enlarged prostate is a condition caused due to presence of

5 α reductase enzyme, hexadecanoic acid and its methyl ester can be a possible tool in mediating and blocking the receptor activity (anti-androgenic). Overall hormonal regulation is carried out by the presence of hexadecanoic acid ester by acting as an intermediate in the synthesis pathway of androgen, estrogen and corticosteroids. Meanwhile, 1,3,7,11,15-tetramethyl-2-hexadecenol was found to have medicinal importance in fighting with certain type of cancer cells, increase the bulk flow of water and salt contents (diuretic action), anti-inflammatory and anti-microbial action [17].

3.3 Nuclear Magnetic Resonance (NMR)

The purified, methanolic extracted compound was analyzed by ^1H NMR and ^{13}C NMR. ^1H NMR was (ppm) 2.474 (3H), 3.135 (1H), 2.5099(1H), 4.0992(1H). The isolated compound was found to be a single analyte present in the extraction identified as 2,3-dihydro-dihydroxy-6-methyl-4H-pyran-4-one (DDMP). This extracted molecule (2,3-dihydro-dihydroxy-6-methyl-4H pyran-4-one) hinders the damage caused by the action of reactive oxygen species (ROS). Furthermore, the ROS consists of superoxide radicals, hydroxyls, and peroxides that result in adverse events and toxicity in healthy cells of human body. This active component shows anti-oxidant activity that is capable of protecting the cell from free radical damage. The pharmacology behind the anti oxidant activity is to hinder the activity of reactive oxygen species that in turn stops growth of cancerous cells [18-19].

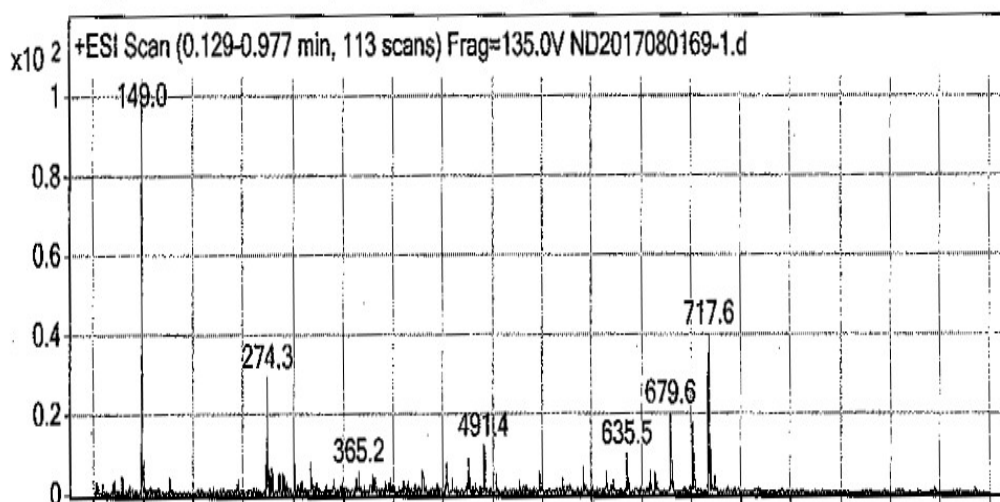


Fig. 5a. +ve LC-MS spectrum of isolated compound of *Urginea wightii* (Benzene)

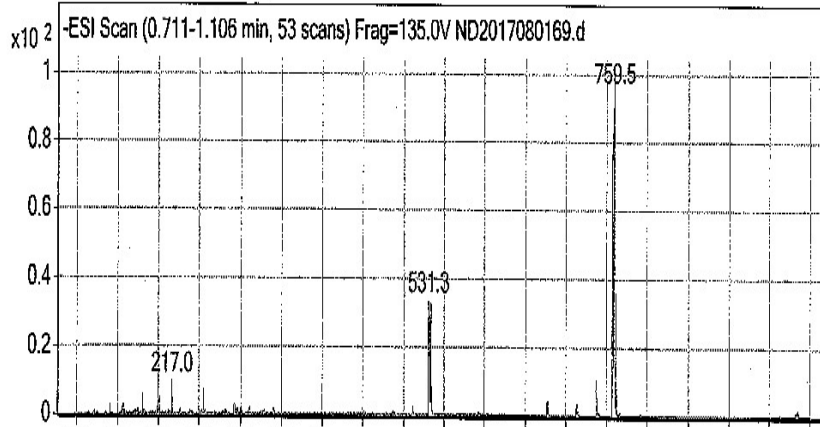


Fig. 5b. -ve LC-MS spectrum of isolated compound of *Urginea wightii* (Benzene)

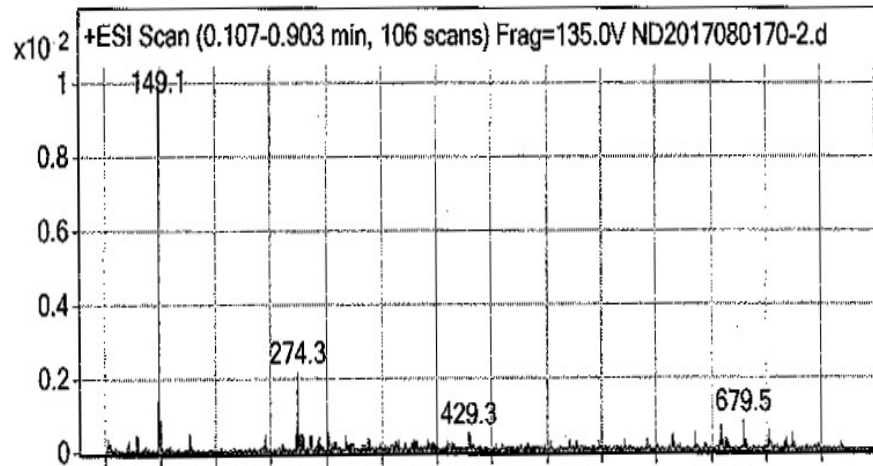


Fig. 6a. +ve LC-MS spectrum of isolated compound of *Urginea wightii* (Benzene: chloroform)

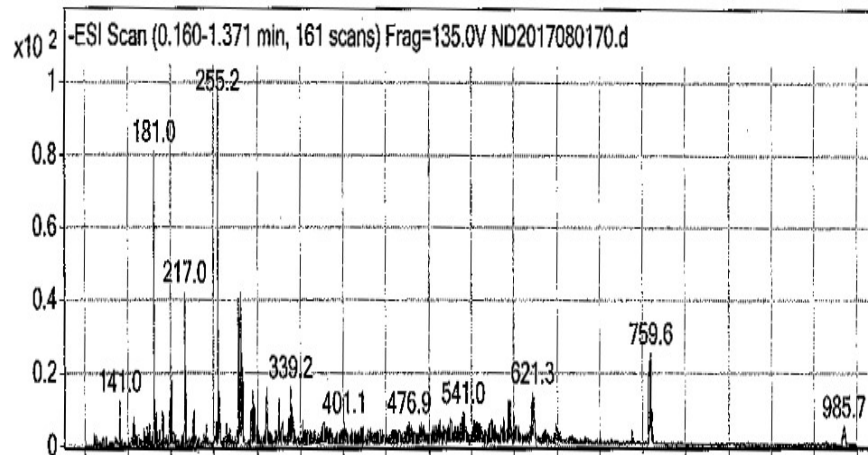


Fig. 6b. -ve LC-MS spectrum of isolated compound of *Urginea wightii* (Benzene: chloroform)

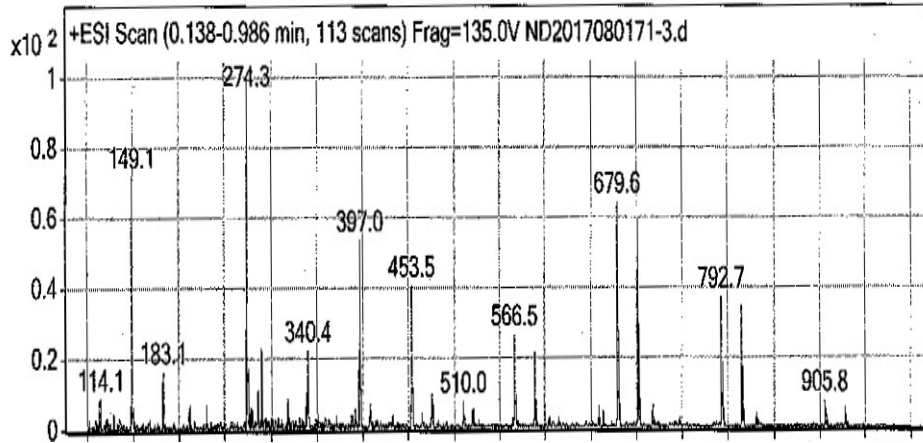


Fig. 7a. +ve LC-MS spectrum of isolated compound of *Urginea wightii* (Benzene: chloroform)

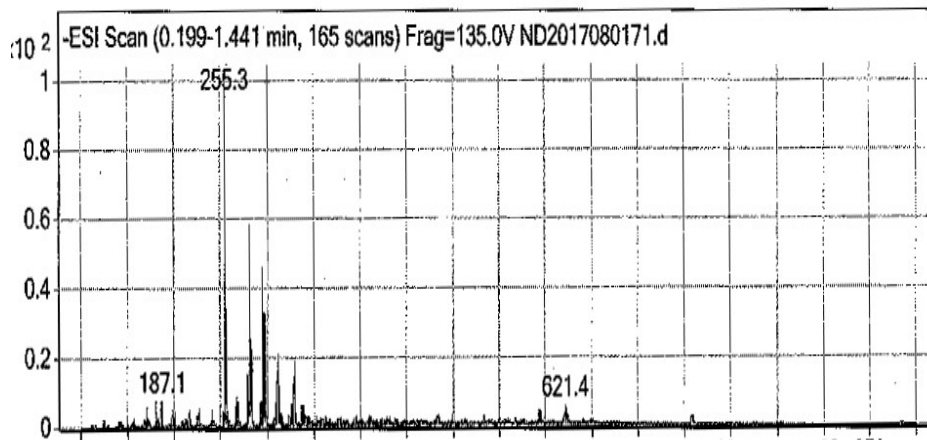


Fig. 7b. -ve LC-MS spectrum of isolated compound of *Urginea wightii* (Benzene: chloroform)

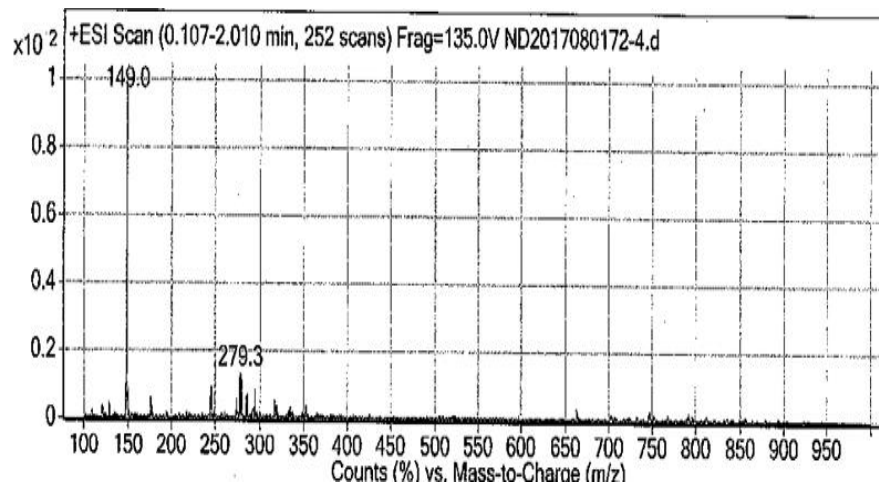


Fig. 8a. +ve LC-MS spectrum of isolated compound *Urginea wightii* (Chloroform)

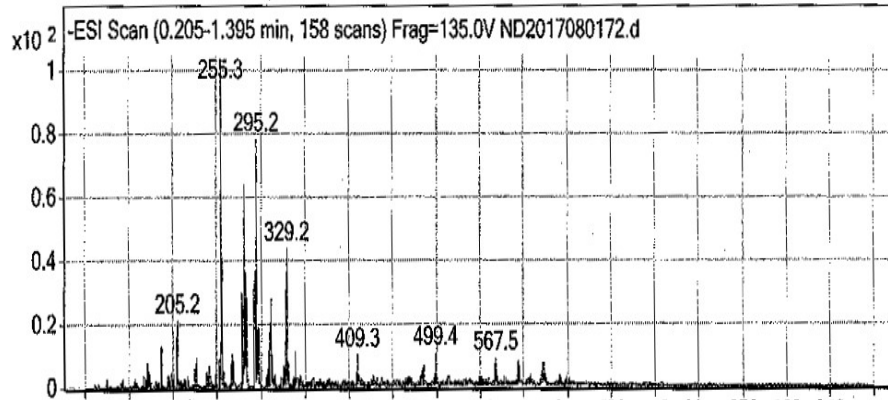


Fig. 8b. -ve LC-MS spectrum of isolated compound of *Urginea wightii* (Chloroform)

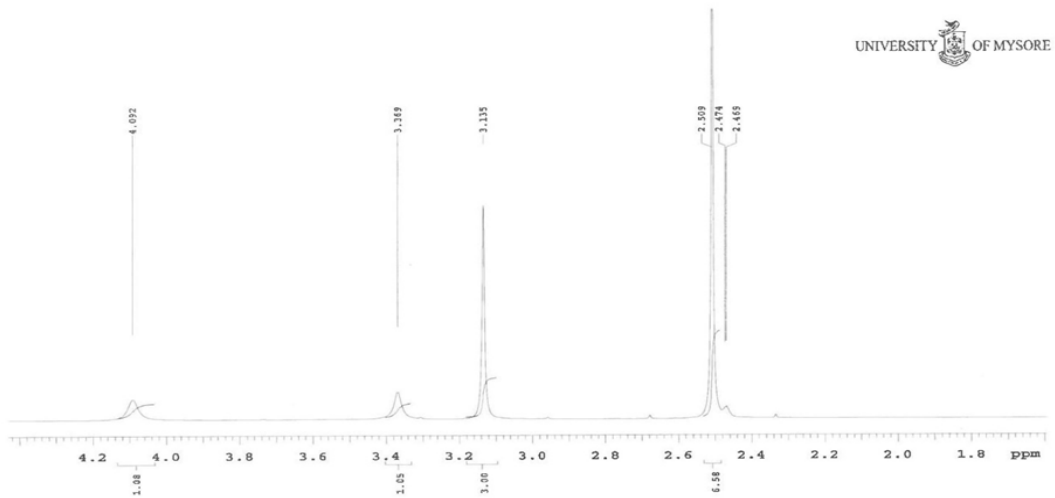


Fig. 9. ¹H-NMR analysis of methanolic extract of *Urginea wightii*

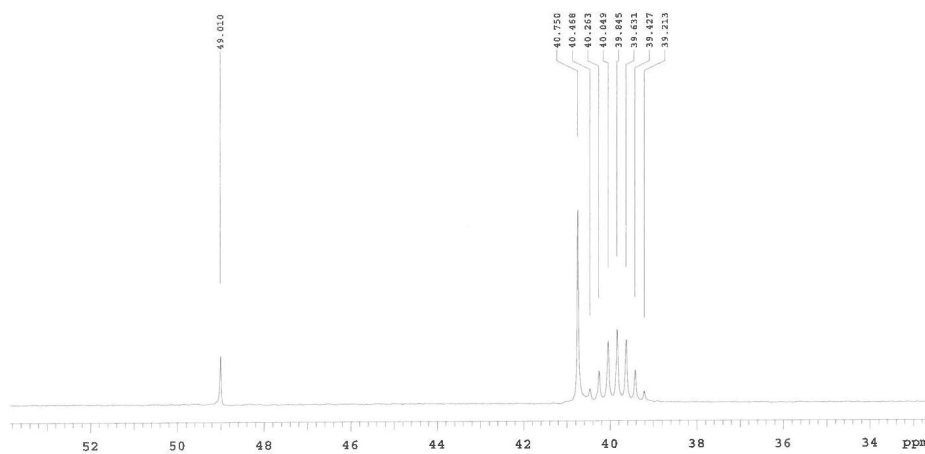


Fig. 10. ¹³C-NMR analysis of methanolic extract of *Urginea wightii*

4. CONCLUSION

Indeed, a considerable attempt is made in this study to identify the active pharmaceutical ingredient from the complex extract of *Urginea wightii*. The scientific data generated from this Phyto- molecular studies using IR, LC-MS and NMR substantiated the proof for use of folklore medicinal system in treating many chronic illnesses in human beings. As the traditional herbal medications are found to be safe for improvising the health in diseased conditions, one can get a major beneficial effect without much untoward reactions. However, there is a need for further quantification of these compounds and development of formulations to deliver it across the human body. This research work will be an initiative in bringing the scientist and health care professionals to a single platform for uncured medical challenges. With this, the intention of our research to make the best possible use of *Urginea wightii* and conserve them for future use will be cleared.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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