

## Isolation of bergenin from the leaves of *Flueggea leucopyrus* willd (katupila) – a novel method of obtaining bergenin.

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### Abstract

Bergenin was isolated as the major active constituent from the leaves of *Flueggea leucopyrus* Willd. The result of this study also provides for the use of *F.leucopyrus* as a novel source for the commercial supply of bergenin to the global market as bergenin is a medicinal agent required for the production of various pharmaceutical formulations used in the treatment of a variety of disorders.

**Keywords:** *Flueggea leucopyrus*, Euphorbiaceae, bergenin

### Introduction

*Flueggea leucopyrus* Willd is a medicinal plant belonging to the family Euphorbiaceae. The leaves of *F.leucopyrus* have been used for treating cancer in the traditional system of medicine in Sri Lanka.<sup>1</sup> However only limited information is available on the active constituents and the pharmacological properties of this plant.

Economical sources for active ingredients are really needed for the large scale production of various dosage forms. Medicinal agents are currently obtained by total chemical synthesis, partial or semi synthesis, genetic engineering or from natural sources viz. plants, animals, microorganisms and minerals. If a drug of natural origin has a complex structure and its chemical synthesis is not economically feasible, its natural sources are still the best sources for obtaining it. A number of clinically useful medicines are still obtained from their natural sources.<sup>2</sup>

Bergenin exhibits a number of interesting pharmacological activities such as anticancer activity, immunomodulatory activity, antioxidant activity and hepatoprotective effects.<sup>3,4</sup> Hence pharmaceutical compositions containing bergenin (eg. compound bergenin tablets) are really useful and important in human and veterinary medicine. Bergenin has traditionally been used as a treatment for bronchial asthma, as an antitussive expectorant, as an anti-inflammatory agent, a protector of the liver and as a treatment for ulcers.<sup>5</sup> Western science validates its use as

anti- obesity agent. Bergenin has proven to be an incredibly effective supplement for people looking to lose weight and keep it off. Not surprisingly, bergenin is becoming a common ingredient in over the counter dietary weight- loss supplements.<sup>6</sup>

Bergenin is currently obtained from different plant species (eg. *Tinospora cripsa*, *Flueggea microcarpa*) to meet the global demands.<sup>7</sup>The present study was carried out to isolate and characterize the major active constituents of *F. leucopyrus*.

## Materials and methods

### Equipment

Infra- Red (IR) spectrum was recorded on a Bruker VECTOR 22 FTIR and Ultra- Violet (UV) Spectrum was recorded on Shimadzu UV Spectrophotometer. Melting point was recorded on SMP 10 Digital melting point apparatus. EI-MS was recorded on MAT 312 mass spectrophotometer. The <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on Bruker NMR Spectrophotometers, operating at 500 MHz The chemical shift values are reported in ppm ( $\delta$ ) units and the coupling constants ( $J$ ) are given in Hz.

### Chromatographic conditions

Precoated aluminium sheets (silica gel G-60F<sub>254</sub>, E. Merck) were used for thin- layer chromatography (TLC). Visualization of the TLC plates was achieved under UV at 254 and 366 nm and by spraying with ceric sulphate reagent. Ethyl acetate (EtOAc)/methanol (MeOH) (9:1) solvent system was used.

### Collection of plant

Leaves and other parts of the plant were collected from Rajangana, Anuradhapura district, in July 2011. Voucher specimens have been deposited in the laboratory of B. Pharm Degree Program, Faculty of Medical Sciences, University of Sri Jayewardenepura. Identification was confirmed by comparison with herbarium specimens housed at Department of Plant Science, University of Colombo.

### Extraction and isolation of bergenin from *F. leucopyrus*

Dried and powdered leaves of *F. leucopyrus* (5 kg) were soaked in 80% methanol H<sub>2</sub>O (10L x 3) at room temperature. The extract was filtered and concentrated under reduced pressure. The concentrated extract (750g) was dissolved in water (1L) and insoluble material was filtered off. The clear aqueous solution was then successively extracted with Hexane (1L x 3), dichloromethane (1L x 3) and EtOAc (1Lx 4). Ethyl acetate extract (200g) was fractionated by Vacuum Liquid Chromatography (VLC) on silica gel and eluted with the mixtures of EtOAc- MeOH to obtain six subfractions. A subfraction eluted with 10-25% EtOAc- MeOH was subjected to column chromatography (CC) on silica gel using gradients of 200ml of EtOAc- MeOH (100% EtOAc, 1% EtOAc- MeOH, 2.5% EtOAc- MeOH, 5% EtOAc- MeOH, 10% EtOAc- MeOH, 20% EtOAc- MeOH, 30% EtOAc- MeOH, 50% EtOAc- MeOH, 100% MeOH) to afford 40 fractions (F<sub>1</sub> – F<sub>40</sub>). Fractions F<sub>27</sub>- F<sub>28</sub> on evaporation yielded a pure compound (20g) designated as JW- FL-1.

**Structure elucidation of JW- FL-I**

The structure of JW-FL-I was elucidated on the basis of its UV spectroscopy, IR spectroscopy, Mass (MS) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy including Distortionless Enhancement Polarization Transfer (DEPT), Homonuclear Shift- correlation spectroscopy (COSY), Nuclear Overhauser Spectroscopy (NOESY), Heteronuclear Multiple- Quantum Coherence (HMQC), Hetero Multiple Bond Connectivities (HMBC) experiments and direct comparison with reported data. JW-FL-I was identified as bergenin by direct comparison of its spectral data with reported data of bergenin. Structure of bergenin is given in Figure 1.

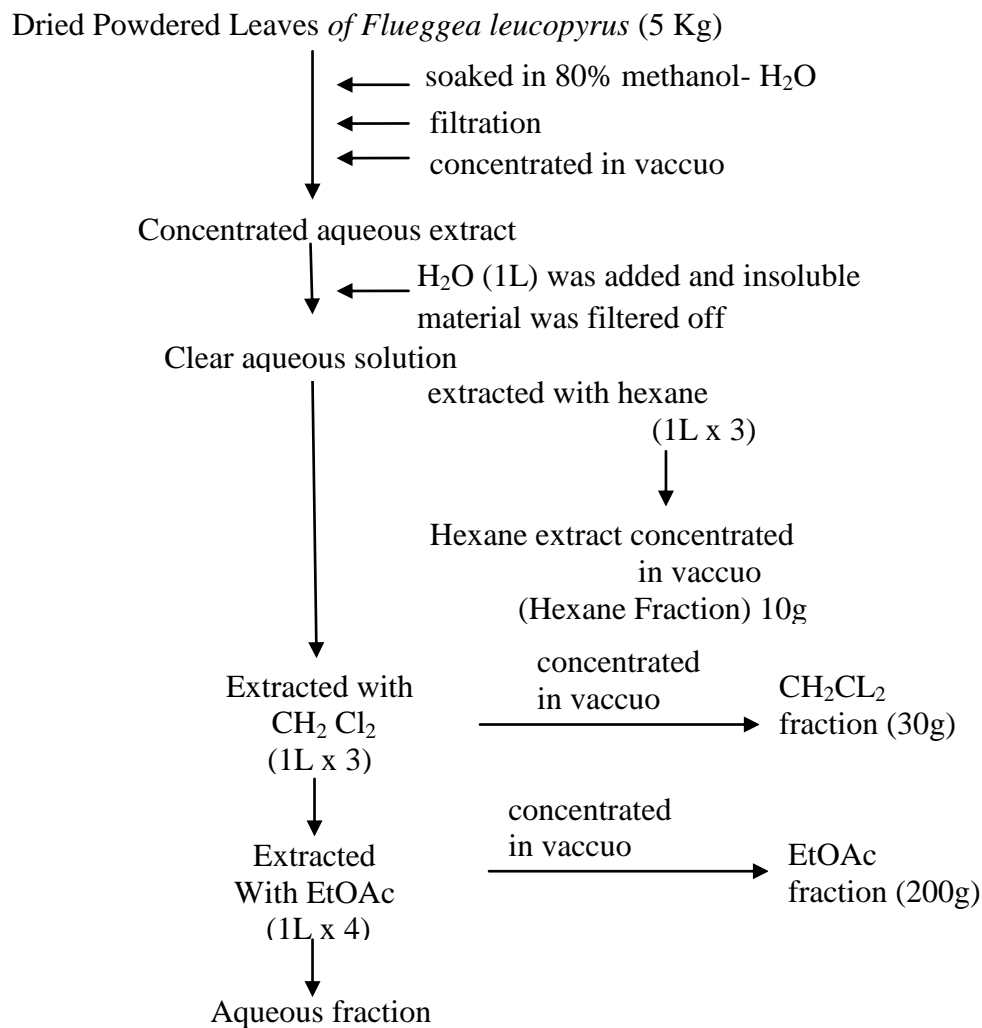
JW-FL-I (bergenin) C<sub>14</sub>H<sub>16</sub>O<sub>9</sub>, Colourless crystals, mp 238°C; EIMS m/z (rel.int.); 328 [M]<sup>+</sup>, 208 (100), 180 (17.2), 179 (14.6), 152 (15.8) and 61 (39.7); UV λ<sub>max</sub> (MeOH) 220 nm, 274 nm; IR ν<sub>max</sub>; 3396 (OH), 1705 (CO), 1344.5 and 1088.4 cm<sup>-1</sup>

<sup>1</sup>H and <sup>13</sup>C NMR data: see Table 1.

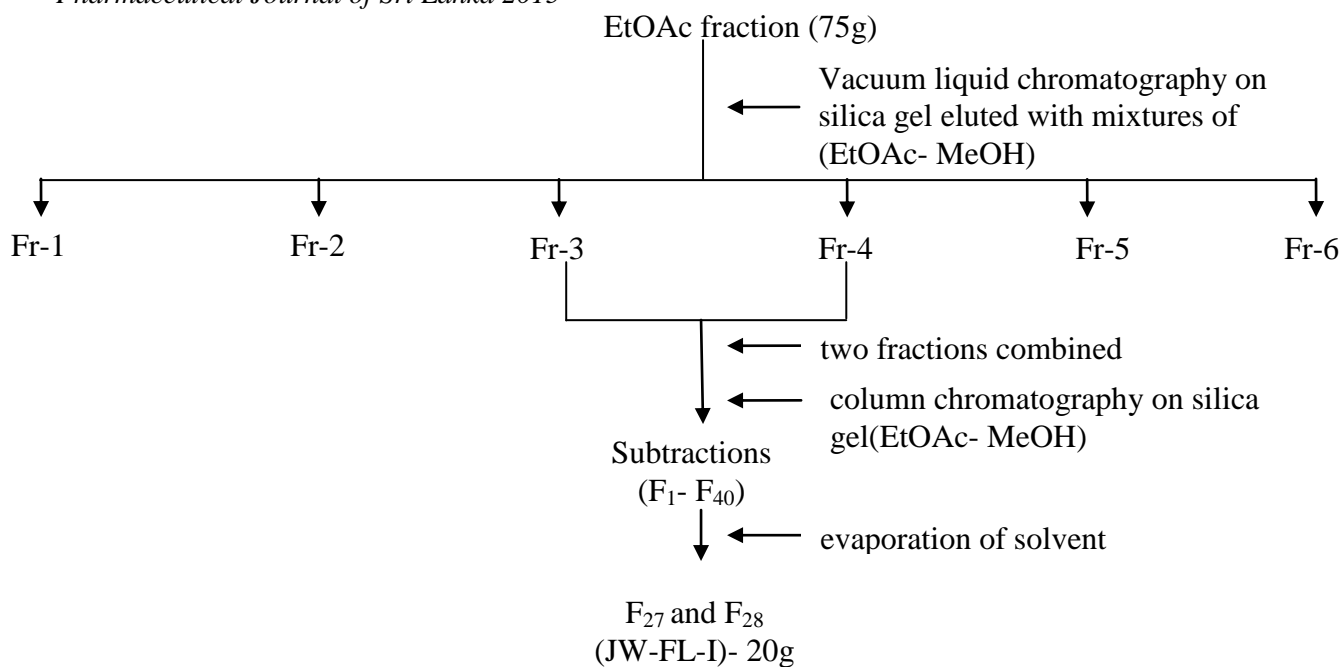
**Table 1:** <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data for JW-FL-1 (bergenin) in CD<sub>3</sub>OD (500 MHz for <sup>1</sup>H and <sup>13</sup>C)

Carbon atom	<sup>13</sup> C (δ)	<sup>1</sup> H, δ (J, H <sub>2</sub> )
1	74.2 (CH)	4.9, d, 10.4
2	83.0 (CH)	3.64, m, 9.1, 7.3, 1.4. 4.6, 6.9, 5.5
3	71.9 (CH)	3.45, dd, 9.0, 8.9
4	75.6 (CH)	3.81, dd, 9.0, 8.9
5	81.4 (CH)	4.04, dd, 10.1, 9.9
6	165.7 (C)	
7	111.07 (CH)	7.06, s,
8	152.2 (C)	
9	142.2 (C)	
10	149.3 (C)	
11	199.4 (C)	
12	117.3 (C)	
13	60.9 (CH <sub>3</sub> )	3.88, s,
14	62.7 (CH <sub>2</sub> )	3.70, dd, 6.9, 4.6

*The <sup>1</sup>H- <sup>13</sup>C Connectivities and <sup>13</sup>C multiplicities were deduced from HMQC and DEPT experiments.*



**Scheme 1:** Extraction and fractionation process for the isolation of bergenin



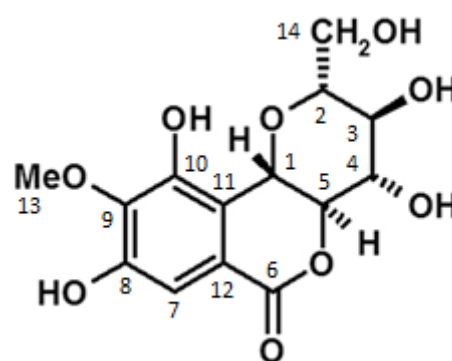
**Scheme 2:** Isolation of bergenin from EtOAc fraction

## Results and discussion

The structure of the major compound JW-FL-1 was determined by spectroscopic analyses and it was identified as bergenin (Figure 1). A significant amount (10-12% on dry weight basis) of bergenin was obtained from the leaves of *F. Leucopyrus* by extracting leaves with 80% MeOH-H<sub>2</sub>O, removing fatty non-polar constituents by partition with ethyl acetate. The ethyl acetate fraction is subjected to VLC on silica gel to obtain fraction enriched with bergenin. This fraction is then subjected to CC on silica gel using gradients of EtOAc- MeOH to obtain very pure bergenin as shown in scheme 1 and scheme 2. The method of obtaining bergenin from *F.leucopyrus* which has not been known so far is introduced to the pharmaceutical industry as a novel method of obtaining bergenin.

## Conclusion

The process of extraction and isolation of bergenin from the leaves of *Flueggea leucopylus* wild could be employed as a



**Figure 1-** Structure of bergenin (1, 3, 4, 4a, 10b, Tetrahydro- 3, 4, 8, 10-tetrahydroxy- 2-(hydroxymethyl)-9-methoxypyran- [3,2-c] [2] benzopyran – 6H- one)

potential method for the commercial supply of bergenin to the pharmaceutical industry.

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