Scientific studies on aerial parts of *Sonchus oleraceus* Linn.

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**Abstract:** *Sonchus oleraceus* (smooth sow thistle) used in various ancient medicinal systems including Chinese medicine. It possesses a wide spectrum of medicinal properties, especially used as an antioxidant, anti-cancer, anti-inflammatory, hepatoprotective, and as anti-aging. This original article has comprehensive laboratory studies on the aerial parts of *Sonchus oleraceus* covering the botanical, physicochemical, macroscopic, microscopic, spectroscopic, phytochemical pharmacological and toxicological aspects in detail. These data will lay the ground for its correct identification and distinguishing it from other *Sonchus* species specially *Sonchus arvensis*. These studies will also be useful to promote its clinical application as an antioxidant and anti-cancer medicine. The data of standardization parameters and distinguishing characters enlisted in this study will ensure the efficacy, safety and will also be helpful for the preparation of a monograph of this weed herb.

**Key Words:** *Sonchus oleraceus*, colon cancer, antioxidant, anti-inflammatory, antinociceptive, anti-aging, flavonoids, microscopic, spectroscopic, gas chromatography mass spectrometric (GC MS) and thin layers chromatographic (TLC) fingerprints.

**Introduction:**

*S. oleraceus* (*S. oleraceus*) species being used in various ancient medicinal systems including Chinese medicine. Whole plant of *S. oleraceus*, especially aerial parts contain a number of pharmacologically active ingredients useful in the treatment of various disease conditions (Bent, S. 2008). *Sonchus oleraceus*, a weed from the family Asteraceae is an annual herb. It is native of Europe, North Africa and West Asia. It has spread to North and South America, India, China and Southern Australia (Chauhan et al., 2006; Holm and Center, 1977)). The genus *Sonchus* comprises of about 60 species and three of them have become common weeds around the world. These are *Sonchus arvensis* (perennial sow thistle) and two are annual species *S. oleraceus* (common sow thistle) and *Sonchus asper*, (spiny sow thistle).*S. oleraceus* used in folk medicines to treat diseases such as enteritis, diarrhea, pneumonia, hepatitis, appendicitis, chronic bronchopneumonia, icterus, throat swelling, hematemesis and uremia.

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(Y.XU, 2005). All parts of the plant are used as a coolant, diuretic, laxative and as a general tonic. An infusion made from the entire plant is taken as a drink. In Baluchistan (Pakistan) the roots and leaves are used as a tonic and febrifuge. The brownish gum left after the evaporation of the juice of this plant is said to be a powerful hydragogue. It has also been used as a so-called cure for the opium habit (Ghazanfar, 1994; Batanouny, 1999). It is also used as anti-cancer, digestive, laxative, emollient, blood purifier and used as liver tonic (Cici, S. et al., 2009) and also reported to have hepatoprotective activity, anti-tumor effect and cardiovascular therapy (LEI Jiand, 200-11).

The organic compounds isolated and elucidated from S. oleraceus are reported as luteolin, luteolin-7-O-β-D-glucoside, apigenin, apigenin-7-O-β-D-glucuronide methyl ester, apigenin-7-O-β-D-glucuronide ethyl ester, apigenin-7-O-β-D-glucopyronoside, apigenin-7-glucoside, germaniclyl acetate, 3-β-hydroxy-6-β-7-α-11-β-H eudesm-4-en-6-12-olide, oleanolic acid and 1-geracerol (Jing–Yu, Liang., et al, 2005). In addition, flavonoids kaempferol, quercetin and their glycoside derivatives were also identified from the whole plant. Caffeic acid, and caftaric acid, ascorbic acid and hydroxyl cinnamic acid have been isolated and identified from the leaf extract (Z-Q, OU., et al., 2013). Loliolide, 15-O-β-glucopyranosyl-11β, 13-dihydrourospermal A, ursoyl acid, lupeol and β-sitosterol-3-O-glucopyranoside have been isolated from the roots of S.oleraceus (Ehab, et al., 2009).

Flavonoids are naturally occurring compounds, as secondary metabolites in the plants functioning as a plant’s physiological survival. They protect against fungal infections and UV radiations (Shashidhra,KV., 2012; Havsteen, Bent., 2002). Apigenins, the most common substances in the flavonoid group have antitumor and anti-carcinogenic activities (Yeung, et al., 2006; Wcislo, Gabriel., et al., 2014; Gupta Sanjay, et al., 2010). Apigenins play most important role in human as anti-cancer: Breast Cancer (Way, TD., et al., 2004), Cervical Cancer (Zheng, et al., 2005), Colon Cancer (Wang, IK., et al., 2000;Botting, et al., 1999), Hematologic Cancer (Wang, IK., et al., 1999) and Lung Cancer (Li, ZD., 2007). Quercetin is one of the important flavonoids, which has anti-carcinogenic property (Vasquez-Garzon, VR., et al., 2009). The prevalence of colorectal adenomas was observed 50% in Maori than in non-Maori New Zealanders from European origin (Dickson, et al., 2010). It was postulated that a specific plant food, which is favored especially by Maori, might offer protection against the onset of colorectal cancers. Sonchus oleraceus (Sow thistle) was found as the most frequently eaten plant food among Maori than the European and pacific island descended New Zealanders (Ferguson et al., 1995; Rush, et al., 2010). Pharmacologically methanol extract of S. oleraceus showed antimutagenic property, possibly leading to Colon cancer prevention (Botting, et al., 1999).

Sonchus oleraceus is well known for its antioxidant activity (A, McDowell., et al., 2011; Z-C,Xia., 2011) which is due to the presence of high concentration of polyphenolic compounds, especially flavonoids. Caftaric acid, Chlorogenic acid and Chicoric acid represent 92 percent of the phenolic compounds in the S.
oleraceous leaves. The hydroethanolic extract of *S. oleraceus* markedly demonstrated antinociceptive (Vilela, al.2009) and anti-inflammatory actions (Vilela, et al. 2010) in rats. *S. oleraceus* extract or its purified constituents could be of potential interest for the treatment of antidepressive disorders (Vilela, et al., 2010).

The biological evaluation of lilolide and 15-O- β-glucopyranosyl-11 β,13-dihydrourospermal A, isolated from the roots of *Sonchus oleraceus* showed cytotoxic activity against PC33 and L5187Y cell lines and also antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *N. gonorrhea* (Ehab, 2009). Leaf extract of *S. oleraceus* showed anti-aging effect (Z, Ou., 2015).

### Material and Methods:

**Collection and identification of plant material:**

The *Sonchus oleraceus* (Figure I) was collected from Al-Mehairbi farm, Liwa, Emirates of Abu Dhabi and identified by Dr. Abdul Nasser Al-Jifri of Zayed Complex of Herbal Research and Traditional Medicine (ZCHRTM), Mafraq, Abu Dhabi, UAE., and preserved as a specimen #1261 at the herbarium of ZCHRTM, Mafraq, Abu Dhabi, UAE.

**Habitat and Distribution:**

It is very common weed of damp habitats in towns, plantations and oases, including industrialized offshore islands; found beside old Abu Dhabi sewage farms. Cosmopolitan. Occurring as a weed of cultivation in Arabian Peninsula. (Ghazanfar, 1994; Western, 1989).

**General appearance:**

The basal leaves are comparatively large and broad. The mature leaves are pinnatifid with irregularly toothed margins, and become increasingly lobed with age. The older leaves form a basal rosette close to the ground, but later-formed leaves are on the flowering stem, which terminates in an inflorescence (Figure I-Aerial Part). The first leaves are orbicular with a slightly serrate margin. The stem and branches are erect, smooth, ridged, and widely hollow and compressed. They are grayish green or brownish yellow with a pinkish tint.

After the appearance of the first floral buds, the internodes become long and the plant height reaches to 1 m or more. Flower heads terminal, yellow. Involucres 10 mm; bracts triangular to lanceolate. Achenes oblanceolate, with pappus.
Figure I: *Sonchus oleraceus* Linn (Family: Asteraceae)

**Common name:** sow thistle, smooth sow thistle, annual sow thistle, Hare’s thistle, Milky tassel.

**Synonyms:** *Sonchus ciliatus* Lam.

*Sotchus australis* Hort. ex Trev.

*Sotchus oleraceus* f. *runcinatus* Fiori.

**Phytochemical studies:**

*Successive extraction with organic solvents:*
The under shade dried plant material was made powder using cutting mill ((Rotor Speed Mill Pulverisette-14-Fritsch-Germany). Accurately weighed 20 grams of the powder was packed in a cellulose extraction thimble and extracted with 250 ml petroleum ether (60-80°C) using soxhlet extraction apparatus. The extraction was continued until the petroleum ether in the soxhlet becomes clear.

After the exhaustive extraction, it was filtered and the solvent distilled off using vacuum evaporator. The obtained residue was dried in a vacuum desiccator over anhydrous sodium sulphate. The yield (%) of the petroleum-dried extract was calculated and stored in a vacuum desiccator.

The left over mark was air dried to remove the solvent completely and was exhaustively extracted with chloroform and absolute alcohol successively. After removing the solvents, the residues were dried in a vacuum desiccator over anhydrous sodium sulphate. The yield of dried extractives were calculated in each case and stored in a vacuum desiccator.

**Preparation of acetone and methanol extractives:**

The acetone and methanol extracts of accurately weighed powder (20 grams in each) were prepared using accelerated solvent extractor system (Dionex ASE 200 accelerated solvent extractor) at a low temperature and high pressure. The solvents were removed and residues dried in freeze dry system (Labconco freeze dry system attached with stoppering tray dryer). The yields of dried powders of acetone and methanol extracts were calculated and stored in a vacuum desiccator for different studies.

**Physicochemical analysis:**

The physicochemical parameters, namely loss in weight on drying, total ash, water soluble and acid insoluble ashes, absolute alcohol and water soluble matter, successive extractives and pH values were determined (WHO, 2011; Evans, 2002).

**Pharmacognostical studies:**

**Macro and Microscopic characteristics:** The arrangements, size, shape, base, texture, margin, apex, venation, color, odor, taste were observed. Microscopic studies were carried out on the thin transverse sections obtained aftercutting by microtome. The photographs of the prepared slides were taken using digital camera fitted on Leica microscope attached to a PC (Tyler V. 1977).

**Thin layers chromatographic fingerprints (TLC fingerprints):** Silica gel 60 F254 coated Aluminum sheets, Merck, Germany were used. HPLC grade solvents were used to prepare mobile phases to develop the thin layer chromatograms (Wagner H, 1996).

Solutions of petroleum ether (60-80) dried extract (50mg/ml) and methanol dried extract (50mg/ml were prepared. 10 microliter of each solution were applied as compact spots (25mm above the base) on the TLC plates using micro syringe.
The developed chromatograms were photographed under UV-254 nm and UV-366 nm (without chemical treatment), and then reprivatized and photographed again under visible and ultra violet lights using CAMAG Video Scan TLC/HPTLC evaluation system, Switzerland.

**GC-MS qualitative analysis of different extractives prepared from dried powder of aerial parts:**

GC-MS-QP2010 Ultra fitted with an auto injector (AOC-20i) system (Shimadzu Kyoto Japan) used for GC-MS qualitative analysis. The system was equipped with mass selective detector with an ion source having temperature 280°C and interface temperature 280°C. Capillary Column used for MS analysis was Rtx 5ms capillary column with 30m x 0.25mm (length and diameter) x 0.25µm film thickness. The temperature of the injector was adjusted to 280°C, selecting injection in split mode. The initial temperature applied was 60°C (2 minutes at hold time), and increased to 180°C (2minutes at hold) and then 300°C at a ramp rate of 9°C and 13°C/minutes respectively. Helium with purity of 99.99% was used as carrier gas with 47.2cm/second of linear velocity. The total flow was 13.3ml/minutes with column flow of 1.69ml/minutes and system pressure was at 100.00Kpa. The all chromatogramswere acquired in scan mode with scan speed1666. The mass range of 40-550 m/z with 1000ev of threshold selected. The mass spectrum of each peak was interpreted based on databases of National Institute Standards and Technology (NIST 11 Lib) and Willey 8 Lib.

**Elemental analysis of ash of the dried powder (aerial parts):** Accurately weighed(5grams) air-dried plant material was completely ashed at about 600°C (BHP, 1996; WHO, 2011). The obtained total ash was dissolved in a known volume of 0.50M Nitric Acid. Ash solution was analyzed quantitatively for different elements present in the plant material using atomic absorption spectrophotometer attached to an auto sampler (AA-6800 Shimadzu Kyoto Japan) in alinement with Hydride Vapor Generator (HVG-1). The flame method was selected for analysis.

**Results:**

**Pharmacognosy & Photochemistry:**

**Plant Material Studied:** Dried leaf.

**Microscopic Characteristics:**

A transverse section through the leaf exhibits its dorsoventrally character (Fig.II A). The upper and lower epidermises consist of oval, somewhat distorted cells that slightly bulge outwards, which are more observed at the lower epidermis. The upper epidermis is underlain by a layer of palisade tissues, which is...
composed of long and broad cells that are compactly packed and they have straight cell walls but some cells have slightly undulating walls. They contain a variety of small-sized colored materials. The spongy mesophyll consists of small rounded cells that are also rich in a mixture of minute colored materials but some cells contain comparatively large brownish yellow masses. Those which enclose the vascular tissues are almost polygonal in shape. The xylem vessels are annularly and spirally thickened.

**Plant Material Studied:** Dried stem and branches.

**Microscopic Characteristics:** A transverse section through the stem or a branch (Fig.II B) exhibits their almost cylindrical outlines with some broad undulations at the outer periphery. The epidermis consists of small oval cells. It is underlain alternatively by two zones: one zone consists of small cortical cells and white unliignified fibers that subtend the isolated groups of vascular tissues while the other zone consists of the cortical cells and unliignified yellowish brown fibers that subtend lignified fibers and cells of wide lumens. The xylem tissues, which are annularly thickened, are moderately lignified. The pith consists of large rounded cells but the cells at the center of the pith are somewhat distorted and they separate from other cells, forming a large circle that surrounds a large hollow area at the very center.

**Parts studied:** Leaf and stem.

**Figure II : (II Ato IIC).**

**Figure II A:** TS of the leaf at the upper portion of the leaf showing the distorted upper epidermal cells with slight bulging; the palisade layer consisting of compactly packed long and broad cells; spongy
mesophyll cells with their contents of colored materials and cells surrounding the vascular tissues are almost polygonal in shape.

**Figure II B:** TS of a portion of the stem showing colorless cortical cells and white un lignified fibers that subtend isolated lignified vascular tissues.

**Figure II C:** TS of a portion of the stem showing cortical cells at the lower left, vascular tissues (dark zone), and the pith zone consisting mainly of distorted compressed parenchyma cells that separate to form a large hollow area (top).

### Physicochemical Constants: (percentage)

Data of physicochemical parameters which have be carried out on the powder of aerial part of the plant *Sonchus oleraceus*, (Quality Control Methods, 2011; Evans, 2002).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying at 105°C</td>
<td>9.60</td>
</tr>
<tr>
<td>Absolute alcohol solubility</td>
<td>5.60</td>
</tr>
<tr>
<td>Water solubility</td>
<td>24.80</td>
</tr>
<tr>
<td>Acetone extractive (Hot extraction)</td>
<td>5.19</td>
</tr>
<tr>
<td>Methanol extractive (Hot extraction)</td>
<td>18.46</td>
</tr>
<tr>
<td><strong>Successive Extractives</strong></td>
<td></td>
</tr>
<tr>
<td>Petroleum ether (60-80 °C)</td>
<td>3.20</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1.70</td>
</tr>
<tr>
<td>Absolute alcohol</td>
<td>15.65</td>
</tr>
<tr>
<td><strong>Ash Values (percentage)</strong></td>
<td></td>
</tr>
<tr>
<td>Total ash</td>
<td>17.33</td>
</tr>
<tr>
<td>Water-soluble ash</td>
<td>11.67</td>
</tr>
<tr>
<td>Acid insoluble ash (10% HCl)</td>
<td>1.33</td>
</tr>
<tr>
<td><strong>pH Values (aqueous solutions)</strong></td>
<td></td>
</tr>
<tr>
<td>pH of 1% solution</td>
<td>5.811-5.813</td>
</tr>
<tr>
<td>pH of 10% solution</td>
<td>5.394-5.405</td>
</tr>
</tbody>
</table>
Table I: Elemental analyses of the ash of the raw powder of the aerial parts:

<table>
<thead>
<tr>
<th>Ash values (British Herbal Pharmacopeia, 1996; WHO, 2011 - References)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay and identification of element (AOAC International - Reference)</td>
</tr>
<tr>
<td>Apparatus (AA-6800 Shimadzu-Flame method)</td>
</tr>
<tr>
<td>Elements</td>
</tr>
<tr>
<td>Cr</td>
</tr>
<tr>
<td>Zn</td>
</tr>
<tr>
<td>Cu</td>
</tr>
<tr>
<td>Fe</td>
</tr>
<tr>
<td>K</td>
</tr>
<tr>
<td>Pb</td>
</tr>
<tr>
<td>Cd</td>
</tr>
<tr>
<td>Ca</td>
</tr>
</tbody>
</table>

1 ppm conc. = 1 µg/ml; Actual conc. (%) = Actual conc. (ppm) x 0.0001 [1 ppm = 0.0001%]

Table II: UV Spectral Studies of methanol extract:

<table>
<thead>
<tr>
<th>Ultraviolet Spectrum (USP reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample conc. (mg / ml)</td>
</tr>
<tr>
<td>1.25</td>
</tr>
<tr>
<td>1.01</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Figure III: Gastric Fluid Simulated without pepsin pH = 1.2 ± 0.1

**Thin layer chromatographic Fingerprints (TLC):** (Reference-Wagner, 1996)

**Figures VI:** (VI A to VID)

TLC fingerprints of petroleum ether (60-80°C) extract (track 1) and methanol extracts (track 2)

- **Mobile phase**
  - Fig. VIA & VID: Toluene, ethyl acetate (93:7)
  - Fig. VIB: Ethyl acetate, methanol, water (100:13.5:10)
  - Fig. VIC: Toluene, ethyl formate, formic acid (5:4:1)
- **Detection:** Fig. VIA: UV 366nm
- **Derivatization:** Fig. VI, Fig. VIB, Fig. VIC, & Fig. D: Vanillin-Sulphuric acid – Vis
GC-MS qualitative analysis result of different extractives of the powder of aerial parts: GC MS chromatograms of Petroleum ether (60-80°C), acetone and methanol extractives of S. oleraceus were qualitatively analyzed. Major compounds namely, pentacosane, ocatadecanal, behenic alcohol, palmitic aldehyde, stigmasterol, methylcommate A, methylcommate B, moretenol, A-neogammacer-en-3-ol, acetate (Fig. V); piperidone, 2, 2, 6, 6-tetramethy, pentadecene, nonadecene, eicosanol and hentetracontanol (Fig. VI) and docosanol acetate, tetracontane, stigmast-5-en-3-ol, (beta), betuline and alpha-selinene (Fig. VII).

Figure V: GC Chromatograph of petroleum ether (60-80°C) extract of Sonchus oleraceus

Figure VI: GC Chromatograph of acetone extract of Sonchus oleraceus
Pharmacological & Toxicological Studies:

Table III: The following pharmacological and safety evaluation studies were carried out on the *Sonchus oleraceus* plant using 70% ethanolic extract (Derelanko 2002; Han, 2003):

<table>
<thead>
<tr>
<th>ACTIVITY</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-diabetic activity</td>
<td></td>
</tr>
<tr>
<td>Analgesic activity</td>
<td>✓</td>
</tr>
<tr>
<td>Anti-inflammatory (ear edema)</td>
<td>✓</td>
</tr>
<tr>
<td>Antidepressant activity (TST)</td>
<td>✓</td>
</tr>
<tr>
<td>Effect on rabbit jejunum</td>
<td>✓</td>
</tr>
<tr>
<td>Effect on rat fundus</td>
<td>✓</td>
</tr>
<tr>
<td>Effect on guinea pig ileum</td>
<td>✓</td>
</tr>
<tr>
<td>Sexual studies (rabbit corpus cavernosum tissue strip)</td>
<td>✓</td>
</tr>
<tr>
<td>Effect on right rat atria</td>
<td>✓</td>
</tr>
<tr>
<td>Acute toxicity studies</td>
<td>✓</td>
</tr>
<tr>
<td>Autonomic effect</td>
<td>✓</td>
</tr>
<tr>
<td>Body weight</td>
<td>✓</td>
</tr>
<tr>
<td>Mortality</td>
<td>✓</td>
</tr>
<tr>
<td>LD_{50} = &gt; 5g/kg p.o.</td>
<td></td>
</tr>
</tbody>
</table>
Summary of the Results:
As shown in the plant extract did not possess any potent anti-hyperglycemic activity; some results suggest the presence of weak spasmodic activity/anti-spasmodic action of the plant extract (Table III). The plant appears to have a significant analgesic activity (Fig. VIII & IX); showed the insufficiency of the sexual response in rabbit corpus cavernous tissue strip method. Significantly reduced the ear edema of the mice (anti-inflammatory) (Fig. IX). Presence of moderate antidepressant activity (Fig. X). Safe at the dose tested; LD$_{50}$ = > 5g/kg, p.o.; no mortality was recorded.

Discussion:
Identification of a plant correctly and then its’ standardization is crucial to maintain its efficacy.
and safety. Parameters for standardization of a crude drug and its extracts include physicochemical constants, macroscopic, microscopic, phytochemical, TLC fingerprinting, and spectroscopic studies. Quantitative elemental analysis of a crude drug is very important along with toxicity tests to determine its safety. Evaluating all these parameters after correct identification of a plant, will ensure and help in maintaining quality, purity, efficacy and safety of the plant as drug.

It is important to evaluate the physicochemical constants for a plant crude drug as they help in identifying adulterants and or improper handling of the plant material. The result of loss on drying was 09.60% indicating that the plant drying process was efficient. This is an important parameter since it determines the efficiency of drying process, which in turn indicative of the stability of the drug storing time. If drying is efficient, it will not encourage the growth of decay causing microorganism. The total ash, water-soluble ash and acid insoluble ash values are indicative of the purity of crude plant. The high value of total ash indicates the presence of inorganic matters and or dusty materials in high concentrations. If acid insoluble ash is low, it is indicative of that a high value of total ash is due to good amount of inorganic elements present in crude drug. High percentage of acid insoluble ash indicates the presence of dusty maters, which in turn indicates that crude plant was not cleaned properly. The total ash, water-soluble ash and acid insoluble ash values are 17.33%, 11.067 and 1.00% respectively. These data indicate that the *Sonchus oleraceus* contains minerals in good concentrations. Extractive values indicate the nature, type and concentration of the chemical constituents present in the crude drug. The extractive value is maximum in water and minimum in petroleum ether. High solubility of chemical constituents of the crude drug in water indicates the presence of high concentration of glycosides, carbohydrates phenolic compounds and inorganic minerals. Ultraviolet spectra (UV spectra), TLC and GC-MS chromatograms of the plant extracts act as fingerprints for it’s identification and as well as identification of chemical constituents present. UV spectra, TLC chromatograms and GC-MS analysis of methanol extract are presented. These data will act as fingerprints for *S. oleraceus*.

Quantitative elemental analysis of the crude plant has been carried out (Table I). These data along with toxicological study results will play a very important role in determining the safety of the crude plant as a drug. Powder, macroscopic, and microscopic studies (Pharmacognostic studies) are essential for every plant, which provide the characteristics of that plant. These data act as reference standards and are considered as diagnostic feature of that particular plant. The results of powder, macro and microscopic studies have been described in detail in the macro and microscopic characteristics parts.

**Conclusion:**

The phytochemical, pharmacognostic, and different spectroscopic data of *Sonchus oleraceus* enlisted in this study will lay the ground for its’ correct identification and will differentiate from the other *Sonchus*
species, especially from *Sonchus asper* as both closely resemble each other. These data will also be useful to promote its clinical application as an aphrodisiac, anti-inflammatory and anti-aging (Life span elongation) medicine.

Results of standardization parameters, quantitative elemental analysis and toxicological & pharmacological data, laid down will ensure the quality, efficacy and safety of the herb as a drug, and will also be helpful in the preparation of a monograph on this weed herb.

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