Pharmacognostical studies of *Smilax aspera* Linn. – A herbal drug

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**Abstract**

**Background:** *Smilax aspera* L. (sarsaparilla or prickly ivy) is a perennial climber from the family Smilacaceae. Its root and rhizome are used as alterative, demulcent, depurative, diaphoretic, diuretic, stimulant and tonic. **Objective:** To perform standardization of root and rhizome of *S. aspera* for authentication and identification of raw drug by pharmacognostical, physiochemical, powder and finish product evaluation. **Materials and Methods:** Air-dried rhizome and roots were boiled, sectioned and stained for macroscopic and microscopic analysis. For physiochemical studies, rhizome and roots were coarsely powdered and subjected for determination of extractive values, ash values, chemical constituents, weight per millilitre, alcohol content, total solids and loss on drying. Finished product analyses (chromatographic studies, sediments, pH and total solid) were also undertaken. **Results:** The root was longitudinally wrinkled, about 3 mm in diameter with numerous branching, rootless, tough and flexible. Cortex consisted of 18–20 rows of parenchymatous cells; xylem and phloem were arranged in a radiate manner. Rhizome was wrinkled, hard and brown externally and white or light-yellow internally. The outer cortex consisted of polygonal parenchymatous cells. The findings of physiochemical determination of raw drugs including maximum extractive values in alcohol were 5.67% w/w, 0.1% w/w foreign matter, 8.90% w/w moisture content, 10.60% w/w total ash, etc., and finished product parameter showed pH near to 7, total solid 1.07% w/v and 50% v/v alcohol content. **Conclusion:** The data represented in this article may be used as distinctive diagnostic characters for proper identification, authentication of raw drug to ensure purity, quality and efficacy of drug *S. aspera*.

**Keywords:** Pharmacognosy, physicochemical, sarsaparilla, *Smilax aspera*, standardisation

**Introduction**

**Smilacaceae** or the Greenbrier family having 350 species in two genera (*Smilax* and *Heterosmilax*) is the second-largest family. The genus *Smilax* is widely distributed in tropical, temperate and subtropical zones and found all over Asia, Europe, Oceania and the USA. Out of 24 species found in India, 4 species, namely *Smilax aspera* Linn., *Smilax perfoliata* Lour., *Smilax wightii* A. DC. and *Smilax zeylanica* Linn., are available in southern India. These species are commonly known as sarsaparilla and mainly present as climbers, with long, thin thorny stem. The stem branches with the help of tendrils attached to other plants or objects to grow steadily upward. All the parts such as rhizome, roots, stems and leaves of sarsaparilla are used in traditional systems of medicine including Ayurveda; in Homoeopathy, mainly its root and rhizome are used. *S. aspera* L., commonly known as sarsaparilla (common milax, prickly ivy or rough bindweed) belonging to the family Smilacaceae, was formerly included in the monocot family Liliaceae. *Smilax comes from ancient Greek language for bindweed*. It is an evergreen, perennial climber with a flexible and delicate stem with sharp thorns. Climber stems reach a height of 1–4 m in woodland or scrub habitats and are sheltered in sharp hooks which also lengthen along the base of the midribs of the leaves. Leaves are dark green, 8–10 cm long, petiolated, alternate, tough, leathery heart shaped and toothed. Yellowish or greenish flowers are very fragrant, small and in axillary racemose, and fruits are 1–3 seeded, red to blue-black coloured berries, 5–10 mm in diameter, rubbery in texture and spherical in...
The seeds are round and bird dispersed. This species is broadly dispersed across the Mediterranean, Central Africa and Southern Europe through Asia into China, Pakistan, India, Sri Lanka, Nepal and Myanmar.[9,11]

Principal chemical constituents present in S. aspera fruit are anthocyanins, pelargonidins and cyanidins. The active constituent is pelargonidin 3-O-rutinoside.[12] Root and rhizome mainly contain steroidal saponins, flavonoids and anthocyanins.[10,12,13] Primary steroidal saponins are (25S)-5b-spirostane-3b-ol-3-O-a-L-rhamnopyranosyl-(1-2)-b-D-glucopyranosyl-(1-2)-b-D-glucopyranoside, curilin G, asparagoside E, asparoside A, asparoside B, (25S)-5b-furostan-1b, 2b, 3b, 5b, 22a, 26-hexaol-26-O-b-D-glucopyranosyl and 26-O-b-D-glucopyranosyl-(25S)-5b-furostan-1b, 3b, 22a, 26-tetraol-1-O-b-D-glucopyranoside.[9,14,15] Seed mainly contains different fatty acids.[10] S. aspera has been used in the treatment of syphilis, rheumatism, stomach pain, bloating, leprosy, psoriasis and diabetes and as an antioxidant to diminish the problems of menopause.[16,17,18,19] The root and rhizome of plant are used as alterative, demulcent, depurative, diaphoretic, diuretic, stimulant and tonic and are also used in the treatment of inflammatory disease, rheumatic arthritis, joint paint and oedema.[20,21]

The present study was, therefore, undertaken to produce standard information for correct identification/authentication of this plant. Even though present modern analytical techniques, for example DNA barcoding, high-performance thin-layer chromatography, high-performance liquid chromatography (HPLC) and infrared, can provide high-quality data for authentication and quality control and quality assurance, here we seek to explore the employment of low-cost yet robust analytical techniques to ensure the quality control and quality assurance of the drug. This information presented in this study may also be consider as the standards ensuring the purity and quality of the drug S. aspera L.

Materials and Methods
The rhizome and roots were obtained from Centre of Medicinal Plants Research in Homoeopathy (CMPRH), Emerald, Ooty (Tamil Nadu), India. Voucher herbarium sample (Herbarium no. 6511) was prepared and preserved at CMPRH, Emerald, Ooty (Tamil Nadu), India. The pharmacognostic and physicochemical parameters were performed as per the protocols mentioned in Homoeopathic Pharmacopoeia of India (HPI).[22] For physicochemical studies, the rhizome and roots were dried in shade. The dried material was powdered coarsely for the preparation of mother tincture.

The chemicals used in the study were double-distilled water, 37% hydrochloric acid (dilution to 10% was done in-house), strong alcohol, chloroform and methanol. All the chemicals used in this study were of analytical grade.

Macroscopic study
Macroscopic characteristics of root and rhizome were noted on the basis of visual observation of raw drug materials as per the prescribed methods.[23,24] It included observation of condition, branching, shape, length, diameter, colour, odour, taste, surface texture and fractures.

Microscopy study
The microscopic study was undertaken by taking appropriate section of the plant parts. Rhizome and roots were boiled separately, cut into small pieces and processed for paraffin method of microtomy as per the method described by Johansen and Wallis.[25,26] Slides of microtome sections cut at 12–15 μm were made, and these slides were further stained with safranin and light green or crystal violet and basic fusion combinations and mounted in Canada balsam and subjected for microscopic observations. Photograph of transverse section of rhizome and roots was taken using Olympus BX53 Research Trinocular Microscope.[27]

Organoleptic characters
Organoleptic characters of powder were evaluated by taking a minute quantity of powder and spread on a white background and visually examined for general appearance, namely nature, colour, odour, taste and texture.

Physicochemical studies
The dried plant material was coarsely powdered and subjected to physicochemical studies which include loss on drying, ash values and extractive values. Mother tincture was prepared following the method described in HPI[22] and subjected for its specific gravity measurement, pH metry, chromatographic profile and ultraviolet (UV) spectroscopy studies.

Thin-layer chromatography
Around 25 mL of mother tincture was heated on a water bath to remove the alcohol. The organics from the aqueous part were extracted using three 25 mL portions of chloroform. The chloroform extract was evaporated to around 2 mL, and thin-layer chromatography was performed using the concentrated chloroform extract using silica gel and chloroform: methanol (90:10) as mobile phase. The spots were detected using UV light of wavelength 365 nm and 254 nm.

Observations and Results
Pharmacognostic studies
Macroscopy
Macroscopic studies revealed that root was longitudinally wrinkled, about 3 mm in diameter, dark reddish-brown colour and with numerous branching rootless, tough, flexible and not breaking easily even when bent double. Rhizome was also wrinkled, hard, externally brown coloured, internally white or light yellow coloured and gave rise to several roots at different points, fracture short, odourless and taste slightly bitter [Figure 1].

Microscopic studies
Root
Transcation of root was circular in outline. Outermost layer epidermis was single layered; epidermal cells were compactly
arranged, polygonal or spherical in shape. Some epidermal cells were modified into root hairs. Epidermis was followed by of 2–4-layered hypodermis. Hypodermal cells were polygonal in shape, tightly packed cells with no intercellular spaces. Hypodermis was followed by multilayered cortex. Cortex consisted of 18–20 rows of parenchymatous cells. These cells were small to large spherical in shape with intercellular space. Endodermis was uniseriate with uniformly thickened walls. Endodermis was followed by a biseriate pericycle layer. Cells of pericycle were smaller in size as compared to endodermis cells. Vascular bundles were radial and polyarch. Xylem and phloem were arranged in radiate manner. Xylem consisted of lignified vessels, with spiral or reticulate thickening and bordered pitted tracheids, fibres lignified, with narrow ends, pitted thickening. Phloem consisted of phloem parenchyma, sieve tubes and companion cells. Large pith was present in the centre composing parenchymatous cells with intercellular space [Figure 2].

**Rhizome**

Transection of rhizome showed a somewhat circular outline. The outermost layer was single-layered epidermis. Epidermal cells were closely arranged, rectangular in shape. Epidermis was followed by hypodermal ground tissue. Hypodermal cells were lignified. Hypodermis was followed by the cortical region which is composed of the outer and inner cortex. The outer cortex consisted of polygonal shape parenchymatous cells; cells contain starch grains, tightly arranged without intercellular space. Inner cortical cells were spherical or oval in shape, loosely arranged without intercellular space; bundles of acicular crystals of calcium oxalate were present. Vascular bundle was collateral, conjoint, closed and scattered. A group of sclerenchymatous fibres was present in inner cortex which also partially covered the vascular bundles. Xylem fibres were of different size, lignified, septate, with pits and large lumen. Xylem vessels were present in small and large size, with scalariform thickening and bordered pitted [Figure 3].

**Powder studies**

The powder microscopy study showed the presence of pieces of epidermal cells and modified root hairs, polygonal hypodermal cell, pieces of cork with groups of tangentially elongated cells; isolated vessels or tracheids either whole or broken and acicular crystals of calcium oxalate.

**Organoleptic characters**

- Colour: Whitish brown
- Touch: Rough
- Odour: Characteristic
- Taste: Slightly bitter.

**Physicochemical studies**

The results of the physicochemical study of the raw drug are described in Table 1. The results of physicochemical studies of raw drugs showed 0.1% w/w foreign matter, 8.90% w/w moisture content, 10.60% w/w total ash, etc., and finished product parameters showed pH near to 7, total solid 1.07% w/v and 50% v/v alcohol content. The loss of drying (LOD) was found to be 8.90%.

In Table 2, the physicochemical data of the mother tincture have been provided. The pH is close to 7. Furthermore, the total solid is 1.07% w/v. This shows that the solvent system is an excellent solvent system for phytochemical extraction. **Preparation of mother tincture**

The mother tincture prepared as per Table 3.
**DISCUSSION AND CONCLUSION**

*S. aspera* is widely used in different medicinal systems for a variety of disorders. The present study demonstrates detailed macroscopic, microscopic, powder studies and physicochemical standards of crude drug and mother tincture of *S. aspera*. The root is longitudinally wrinkled, about 3 mm in diameter with numerous branching rootless, tough and flexible. Rhizome is also wrinkled, hard and externally brown colour, internally white or light yellow colour. Microscopic studies show the unique arrangements of epidermal, hypodermal, cortex and vascular tissue in root and rhizome of *S. aspera*. In physicochemical parameters, LOD percentage provides the information regarding the amount of raw wet drug required for the mother tincture preparation. The total ash was found to be around 10%. This value suggests that the metallic component in the raw drug is quite low. The acid-insoluble ash is <5%. This indicates that the amount of silicates in the raw drug is moderate. This is quite expected from the fact that the part for the drug used is exclusively sourced from the underground part of the plant. The pH of the mother tincture is close to 7, indicating that the mother tincture is safe for oral administration. Here, we have shown that even without using high-end instruments this study has been carried out, and we hope, this will pave the path for simple yet robust drug standardisation research. However, considering the new age instrumentation advances in analysis, for example HPLC-mass spectrometry (MS), gas chromatography-MS and nuclear magnetic resonance, we will also seek to explore the use of these modern analytical instruments in the future. This will provide a comparison of our present study with future studies employing modern analytical instruments. We are hopeful that these data may also be considered as pharmacopoeial standards for the drug *S. aspera*.

**Acknowledgement**
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Nil.

### Table 1: Raw drug parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreign matter</td>
<td>0.1% w/w</td>
</tr>
<tr>
<td>Moisture content (L.O.D. at 105°C)</td>
<td>8.90% w/w</td>
</tr>
<tr>
<td>Total ash</td>
<td>10.60% w/w</td>
</tr>
<tr>
<td>Acid-insoluble ash</td>
<td>4.28% w/w</td>
</tr>
<tr>
<td>Water-soluble ash</td>
<td>1.07% w/w</td>
</tr>
<tr>
<td>Extractive values</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>5.67% w/w</td>
</tr>
<tr>
<td>Distilled water</td>
<td>14.11% w/w</td>
</tr>
</tbody>
</table>

### Table 2: Finished product parameters

<table>
<thead>
<tr>
<th>Parameter and Observations</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organoleptic profile</td>
<td></td>
</tr>
<tr>
<td>Appearance</td>
<td>Clear, non-viscous</td>
</tr>
<tr>
<td>Colour</td>
<td>Sunset yellow</td>
</tr>
<tr>
<td>Odour</td>
<td>Woody</td>
</tr>
<tr>
<td>Sediments</td>
<td>Absent</td>
</tr>
<tr>
<td>Weight per milliliter</td>
<td>Not &gt;0.95 g</td>
</tr>
<tr>
<td>Total solids</td>
<td>Not &lt;1.07 % w/v</td>
</tr>
<tr>
<td>Alcohol content</td>
<td>50 % v/v</td>
</tr>
<tr>
<td>pH</td>
<td>6.33</td>
</tr>
<tr>
<td>λ-max</td>
<td>232, 278 nm</td>
</tr>
<tr>
<td>TLC</td>
<td>Mobile phase: Chloroform:methanol (9:1 v/v)</td>
</tr>
</tbody>
</table>

### Table 3: Preparation of Mother tincture

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother tincture (φ)</td>
<td>1/10</td>
</tr>
<tr>
<td>Drug strength</td>
<td></td>
</tr>
<tr>
<td>Sarsaparilla in coarse powder</td>
<td>100 g</td>
</tr>
<tr>
<td>Purified water</td>
<td>500 mL</td>
</tr>
<tr>
<td>Strong alcohol</td>
<td>537 mL</td>
</tr>
<tr>
<td>To make 1000 mL of the mother tincture</td>
<td></td>
</tr>
<tr>
<td>Potencies: 2× to contain one part mother tincture, four parts purified water, five parts strong alcohol; 3× and higher potencies to be prepared with dispensing alcohol</td>
<td></td>
</tr>
</tbody>
</table>
Conflicts of interest
None declared.

REFERENCES


**Études pharmacognostiques de Smilax aspera Linn - Un médicament à base de plantes**

**Contexte:** Smilax aspera L. (Salsepareille ou lierre de Barbarie) est une plante grimpante vivace de la famille des Smilacaceae. Sa racine et son rhizome sont utilisés comme altérant, adoucissant, dépuratif, diaphorétique, diurétique, stimulant et tonique.

**Objectif:** Standardiser la racine et le rhizome de S. aspera pour l’authentification et l’identification du médicament brut par évaluation pharmacognostique, phytochimique, en poudre et du produit fini. **Matériel et méthodes:** Le rhizome et les racines séchées à l’air ont été bouillis, coupés et colorés pour une analyse macroscopique et microscopique. Pour les études physico-chimiques, le rhizome et les racines ont été grossièrement pulvérisés et soumis à la détermination des valeurs d’extraction, des valeurs de cendres, des constituants chimiques, du poids par litre, de la teneur en alcool, des solides totaux et de la perte au séchage. Des analyses de produits finis (études chromatographiques, sédiments, pH et solide total) ont également été entreprises.

**Résultats:** La racine était plissée longitudinalement, d’environ 3 mm de diamètre avec de nombreuses ramifications, sans racines, résistantes et flexibles. Le cortex se composait de 18 à 20 rangées de cellules parenchymateuses; le xylème et le phloème ont été disposés de manière rayonnée. Le rhizome était ridé, dur, brun à l’extérieur et blanc ou jaune clair à l’intérieur. Le cortex externe était constitué de cellules parenchymateuses polygonales. Les résultats de la détermination physico-chimique des drogues brutes, y compris les valeurs d’extraction maximales dans l’alcool, étaient de 5,67% p/p, 0,1% p/p de matières étrangères, 8,90% p/p de teneur en humidité, 10,60% p/p de cendres totales, etc., et fini le paramètre du produit a montré un pH proche de 7, un solide total de 1,07% p/v et une teneur en alcool de 50% v/v. **Conclusion:** Les données représentées ici peuvent être utilisées comme caractères de diagnostic distinctifs pour une identification correcte, l’authentification du médicament brut pour assurer la pureté, la qualité et l’efficacité du médicament S. aspera.
Pharmacognostical Studien von Smilax aspera Linn - Eine pflanzliche Droge


Ergebnisse: Die Wurzel war längs faltig, etwa 3 mm im Durchmesser, mit zahlreichen Verzweigungen, wurzellos, zäh und flexibel. Cortex Bestand von 18-20 Zeilen von parenchymatösen Zellen; xylem und phloem angeordnet, die in einem strahle Art und Weise. Rhizom war faltig, hart, Braun und außen und weiß oder hell - gelb intern. Die äußere Rinde Bestand aus polygonalen parenchymatösen Zellen. Die Ergebnisse der physikalisch-chemischen Bestimmung von roh-Drogen, einschließlich maximale mineralgewinnenden Werte in Alkohol wurden 5.67% w/w 0,1% w/w Fremdkörper, 8.90% w/w den Wassergehalt 10.60% w/w Gesamt-Asche, usw., und fertigen Produkt-parameter zeigten pH-Wert in der Nähe von 7, insgesamt solide 1.07% w/v und 50% v/v Alkohol.

Fazit: Die Daten dargestellt , die hier verwendet werden können, wird als eindeutiges diagnostisches Zeichen für die korrekte Identifizierung, Authentifizierung von roh-Drogen, um sicherzustellen, Reinheit, Qualität und Wirksamkeit von drug - S. aspera.

斯米拉克斯阿斯佩拉林的药理学研究- 一种草药

背景：斯米拉克斯阿斯佩拉 L.（萨萨帕里拉或刺常春藤）是一个常年登山者从家庭斯米拉卡塞。其根和根用于替代，去尿，净化，抗药性，利尿，兴奋剂，补品。目的: 通过药理学、物理化学、粉末和成品评价，对原药进行根和根部标准化。对原药进行鉴定和鉴定。材料和方法：风干根部和根被煮沸，-formal和染色，用于宏观和微观分析。在物理化学研究中，根部和根部经过粗糙处理，用于测定萃取值、灰值、化学成分，每毫升重量、醇精含量，总固体和干燥损失。还进行了成品分析（色谱研究、沉积物，pH值和总固体）。结果：根部纵向起皱，直径约3毫米，有许多分支，无根，坚韧和灵活。皮层包括18-20行的实质细胞；木质部和韧皮部被安排在辐射的方式。根茎起皱，硬，外部为棕色，内部为白色或淡黄色。外层皮层由多边形实质细胞组成。原药物理化学测定结果（包括醇精中最大浓度值）为5.67% w/w，0.1% w/w异物，8.90% w/w水分含量，10.60% w/w总灰，成品参数显示pH值接近7，总固体1.07% w/v和50% w/v醇精含量。结论：此处显示的数据可用作正确识别、鉴定原药的独特诊断特征，以确保药物S. aspera 的纯度、质量和疗效。