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PHARMACOGNOSTICAL STUDIES ON ROOTS OF *SAUSSUREA COSTUS* (FALC.) LIPSCH.

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The plant selected for the present study was *Saussurea costus* (Falc.) Lipsch, which belongs to Asteraceae family. *S. costus* roots are used traditionally to cure a variety of conditions, including infection in the throat, dysentery, ulcers, and asthma. Studies have shown that *S. costus* roots have a wide range of therapeutic effects, including anti-oxidant, anti-tumor, hepatoprotective, and anti-inflammatory. In the present study, a pharmacognostical evaluation was done to provide supplementary information on the identification parameters of *S. costus*. This evaluation includes examination of morphological and microscopical characters; fluorescence studies; determination of physicochemical parameters; and phytochemical screening. Qualitative chemical detection of inulin in root powder was also carried out. The microscopical study found the presence of resin ducts, xylem vessels, inulin crystals, cork cells, tracheids, and fibres. The photomicrographs of microscopical elements are displayed in this study. The preliminary phytochemical screening of the root of *S. costus* revealed the presence of terpenoids, flavonoids, alkaloids, tannins, and many other constituents. The current pharmacognostical assessment of *S. costus* roots can be successfully used in laboratory and manufactory operations for the identification of plant powder and detection of adulteration, which are considered important parameters for the plant prior to its usage.

Keywords: *Saussurea costus*, Asteraceae, Root, Inulin, Macroscopic characters, Microscopical examination, Phytochemical screening.

INTRODUCTION

Since the dawn of time, people have employed plants to treat a wide range of diseases. To this day, various ailments are treated with plant decoctions. Herbal plants are very valuable not only for their therapeutic properties but also for their economic value in that they provide the pharmaceutical industry with raw materials and active compounds. Our present study is based on a potential perennial herb from the Asteraceae (Compositae) family, which is called *Saussurea costus* (Falc.) Lipsch. (*S. costus*). *Costus* is one of the best-known species within the *Saussurea* genus because of its greatest medicinal and economic viability among all *Saussurea* species. In the Ayurvedic, Chinese, and Tibetan systems of medicine, *S. costus* is well-documented for its therapeutic abilities, and it is one of the principal constituents in around 175 formulations listed in The Handbook of Traditional Tibetan Drugs[1]. *S. costus* exists in literature under synonyms such as *Aucklandia lappa* and *Saussurea lappa* (*S. lappa*). It has also become well-known in the past 2500 years

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and has been used by traditional healers since the beginning of Islamic civilisation in Arab countries under the names Al-Kost Al-Hindi, Al-Kust, and Al-Qust.[2]. *S. costus* has a variety of various vernacular names, typically referred to as Kuth or Kustha in India[1], and as kuth or costus root in English[3]. *S. costus* is a native of China, Pakistan, and India, and it spreads extensively throughout the Himalayan region, growing at an altitude of 2500–3500 m[4]. Some *Saussurea* species are well known for their medicinal and economic worth. Accordingly, because of their great medicinal and commercial value, they have been extensively used and exhausted. *S. costus* is one of 37 endangered medicinal plants from the Himalayas that have been prioritised for *in-situ* and *ex-situ* conservation because the impact of exploitation has become so severe that it is classified in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)[5]. Indigenous people used dried roots, which contain essential oil[1], for ethnomedical purposes, which are listed in Ayurveda, Unani, and Siddha, and are exploited by the pharmaceutical industry[2]. *S. costus* has long been used in medicine without causing any obvious side effects[4]. *S. costus* roots are used to cure a variety of conditions, including infection in the throat, dysentery, ulcer, toothache, asthma, stomach ache, cough and cold[6], typhoid, piles, headache, and rheumatism and painful joints[1]. Many *in-vitro* and *in-vivo* pharmacological studies have clearly shown that *S. costus* root and root oil exhibit a wide range of therapeutic effects, including anti-oxidant, anti-microbial[6], anti-tumor, anti-fungal[1], hepatoprotective, anti-inflammatory, and used in the treatment of bronchitis[5]. Several chemicals isolated from this plant have therapeutic effects, including costunolide, dehydrocostus lacton[4], alantolactone, cynaropicrin[1], and saussureamines[6]. Recent studies on *S. costus* suggest that it might be useful for treating SARS-CoV-2[2]. That may be due to lupeol, a terpenoid that exists in *S. costus* and targets the coronavirus spike protein. *In-silico* study, lupeol was reported to have a high affinity for the spike (S) protein[7]. Additionally, *S. costus* functions as an immunomodulating agent. Its immunomodulating activity due to curcumene[8], inulin[9], costunolide, dehydrocostus lactone, and guaianolides[1]. As a result of all of this, *S. costus* is now a significant drug on the global market, so its demand is rising[5]. Due to this increasing need, this endangered Himalayan species is frequently replaced by other morphologically similar species, whether knowingly or unknowingly. One such plant frequently discovered in the market samples of "Kuth" is *Arctium lappa* (*A. lappa*), which is a member of the same family. However, there are some key microscopic characteristics that allow *S. costus* roots to be distinguished from *A. lappa* roots, such as the absence of the schizogenous resin ducts in *A. lappa* roots[10]. Therefore, the study of microscopical characters of *S. costus* is crucial for the detection of adulteration and distinguishing between *S. costus* and other species that have similar morphology. In this study, we carried out a detailed pharmacognostical studies on a sample of *S. costus* root from the Egyptian market.

MATERIAL AND METHODS

Plant material

The plant material was obtained from a spice shop (Al Attar House) in Assiut City, which is a governorate in Upper Egypt. The plant root was purchased in its entire form and also in a fine powdered state. A specimen of *S. costus* root (entire and powder) under number SC1 was deposited in the Pharmacognosy department, Faculty of Pharmacy at Sphinx University, New Assiut City, Assiut, Egypt.

Macroscopical analysis

The morphological and organoleptic characteristics of the dried root of *S. costus* were evaluated as per literature by observing its shape, size, colour, odour, taste, and different features like fracture and texture[11-14].

Microscopical analysis

The powder and scraping samples of *S. costus* root were observed with mounts like water, glycerin, KOH, and phloroglucinol with

hydrochloric acid for the study of microscopical characters. The microscopical examination was carried out by Optika B-159 light microscope, and the photomicrographs were taken using Optika advanced biological microscope with Optika C-B10 digital camera. Also, the software that has been used is Optika vision lite 2.1.

Chemical characteristic colour reactions and fluorescence studies

Many crude medicines show fluorescence when the specimen is exposed to UV light. It is uncommon to perform crude drug evaluation based on fluorescence in daylight because the fluorescence impact is typically faint and inaccurate. Fluorescence lamps, which are fitted with appropriate filters and transmit UV radiation of a specific wavelength, eliminate visible radiation from the lamp. If the substances are not fluorescent by themselves, they can frequently be transformed into fluorescent derivatives by using various reagents. As a result, some crude drugs are often evaluated qualitatively in this manner, and it is an important point in pharmacognostical evaluation[15]. The powdered plant material of *S. costus* was treated using a variety of chemical agents. After two hours, they were exposed to visible and ultraviolet (UV) light to examine the fluorescence behaviour of the samples. The appearance and colour shown in visible and ultraviolet (UV) light were noticed and recorded. According to the American Herbal Pharmacopoeia[16] and other recent studies[10], *Saussurea* radix has inulin crystals. There are some tests that are listed in the pharmacopoeia that can be carried out to determine the presence of inulin[17-19].

Physicochemical parameters

A powder of the dried roots of *S. costus* was used for determining some physicochemical parameters such as foaming index and extractive values according to the standard procedures recommended in WHO guideline of quality control methods for medicinal plant materials[11].

Preliminary phytochemical studies

Preliminary phytochemical screening was done on an different extracts of the dried roots of *S. costus* to determine the presence of several phytoconstituents such as monoterpenes, triterpenes, sesquiterpenes, alkaloids, flavonoids, glycosides, lignans, saponins, anthraquinones, steroids, tannins, coumarins, and many other constituents using standard protocols described by Kokate and Harbone[20-22].

RESULTS AND DISCUSSION

The medicinal plant must be thoroughly researched before being used because the therapeutic efficacy is totally dependent on the quality of the plant material employed. The original and fundamental approach to pharmacognostical studies involves morphological study, microscopic study, and preliminary phytochemical screening, which still plays an important role in identifying the proper species of plant and also in distinguishing between closely related species of the same genus. Additionally, it is the initial stage in standardising medicine, which is currently necessary. In the present study, a detailed pharmacognostical investigation of a sample of *S. costus* root from the Egyptian market was carried out.

Morphological (Macroscopical) characters

Dried roots of *S. costus* (Falc.) Lipsch. are cylindrical or semi-cylindrical, about 12 cm long and 0.6-2.3 cm in diameter and have longitudinal distinct wrinkles. The dried roots are very hard and their fracture is short and their texture is rough. Its organoleptic characteristics are dark yellowish brown in color, characteristic in odour, and bitter and pungent in taste as shown in figure 1.1. Plant's specimens from the Royal Botanic Gardens, Kew K000372736[23] and K000372737[24] as well as its habit and its inflorescence from garden of medicinal plants of Royal College of Physicians (RCP)[25] shown in figure 1.2.

Fig. 1.1: Dried roots of *Saussurea costus* (Falc.) Lipsch. from the Egyptian market (x1).

Fig. 1.2: *S. costus* specimens, its habit, and its inflorescence. (https://garden. rcplondon.ac.uk/plant/ Details/1154).

Microscopic characters

The microscopical examination of *S. costus* root powder showed some important microscopic elements, including the presence of schizogenous resin ducts and fragments of orange or brownish resin canals (Fig. 2.1). Elongated or drum-shaped xylem vessels associated with spiral scalariform and reticulate secondary wall thickening (Fig. 2.2). Amorphous and colourless inulin masses were present with different shapes (Fig. 2.3). However, inulin is a polysaccharide in nature, has a good crystalline formation in alcoholic solution. Inulin is widely distributed as the primary storage substance in the roots of Asteraceae plants[16]. Fragments of brown polygonal cork cells were present (Fig. 2.4). In addition, tracheids are typically found in groups or singly and are rectangular, elongated cells with numerous conspicuous pits (Fig. 2.5, p-q), whereas tracheidal fibers are thinner and longer with acute apexes and have numerous slit-shaped pits (Fig. 2.5, r). Also, the presence of many lignified long fibers with narrow to broad lumen associated with pointed ends (Fig. 2.6).

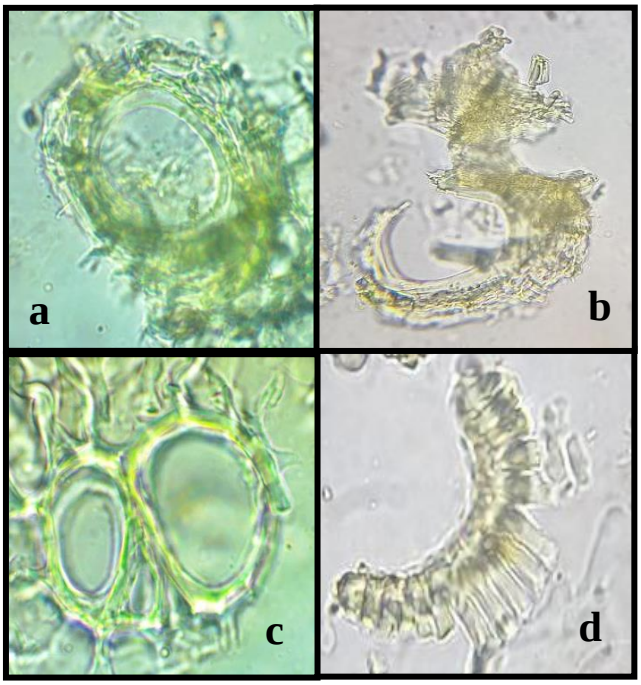

Fig. 2.1: Schizogenous resin ducts (a-d x40).

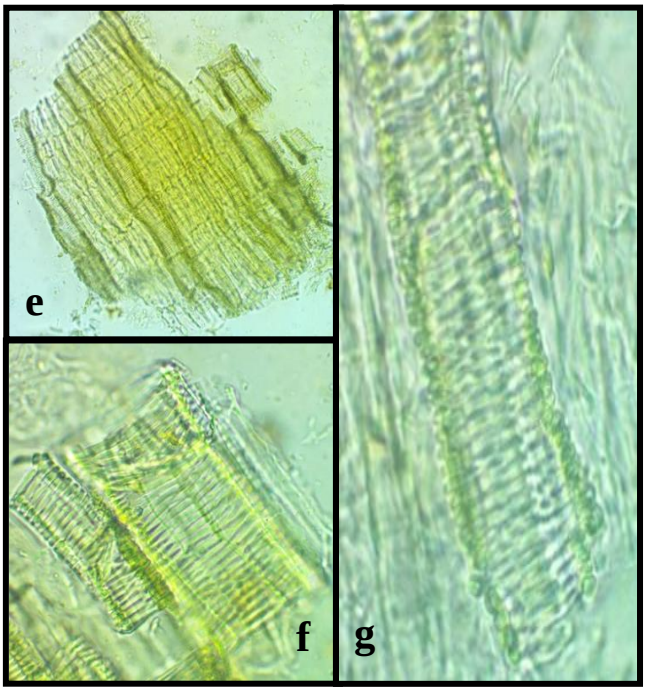

Fig. 2.2: Xylem vessels (e x10, f-g x40).

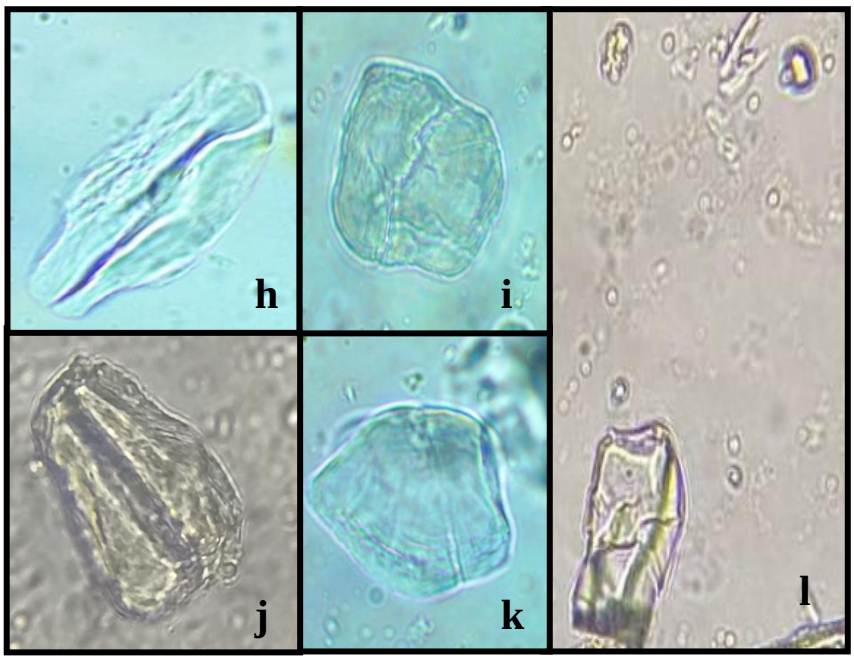

Fig. 2.3: Inulin crystals (h-l x40).

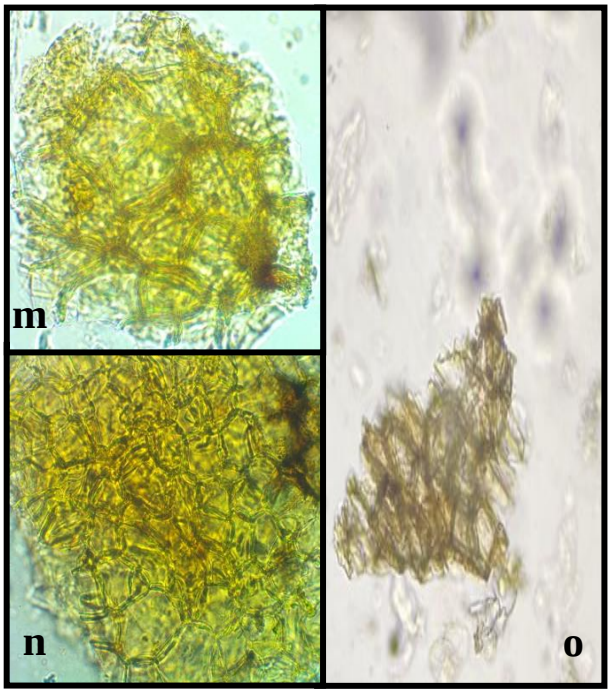

Fig. 2.4: Cells (m-n x40, o x10).

Fig. 2.5: Tracheids (p-q x40) and tracheidal fiber (r x40).

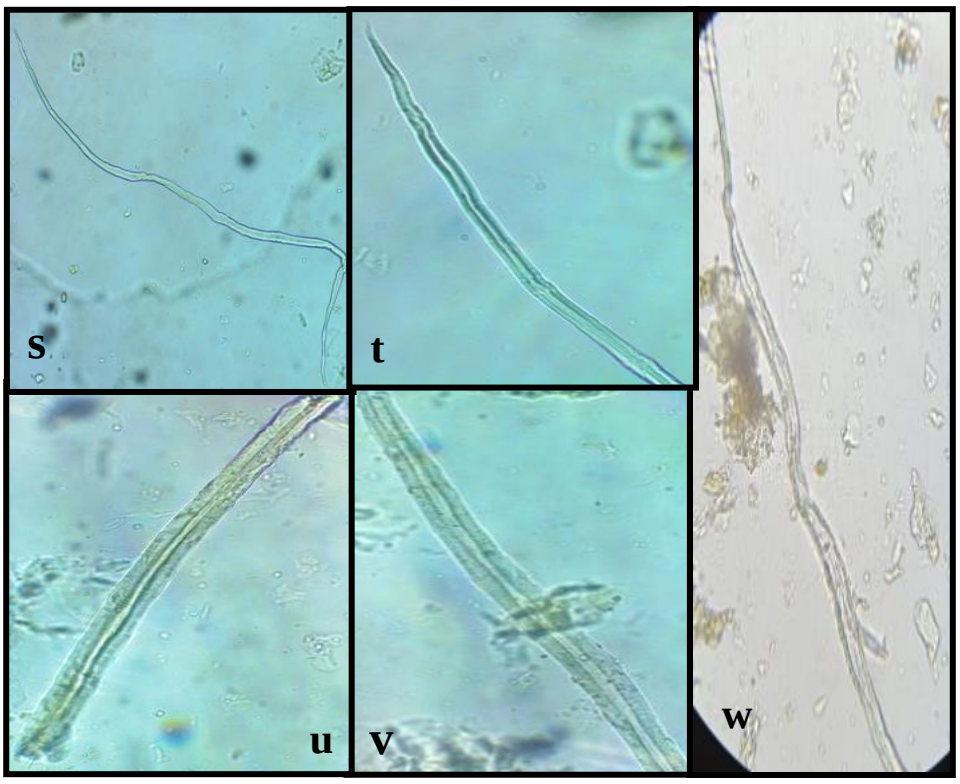

Fig. 2.6: Fibers (s x10, t-w x40).

Chemical characteristic colour reactions and fluorescence studies

The reaction with iodine to detect starch in root powder was carried out with an aqueous solution of iodine. As a result, the characteristic blue colour did not appear, which indicates the absence of starch. The qualitative detection of inulin reaction using resorcinol solution has been done. 1-2 drops of resorcinol solution and 1 drop of concentrated sulfuric acid are added to the root powder, an orange-red colour was appeared, which indicates the presence of inulin (Fig. 3). The presence of inulin can be observed only in the absence of starch[17]. Another pharmacopeial test was reported with 2-3 drops of thymol alcohol solution (20%) and 1 drop of conc. sulfuric acid. Root powder will be coloured with an orange-red stain due to inulin[18&26] or 1-2 drops of α-naphthol alcohol solution (20%) and 1 drop of conc. sulfuric acid; appears reddish violet staining[17]. The powder of dried *S. costus* roots was treated and kept separately in a variety of chemical agents for two hours before their characteristic colours in visible light were observed and recorded in table 1. Also, the fluorescence of their filtrates was studied under UV light, and the results were summarized in table 2.

Physicochemical studies

Extractive values are most frequently used to identify exhausted or adulterated plants. The water, methanol, chloroform, and diethylether soluble extractive values have been listed in table 3. To determine the foaming index, the powder of *S. costus* dried roots was shaken with water and did not foam (Table 3).

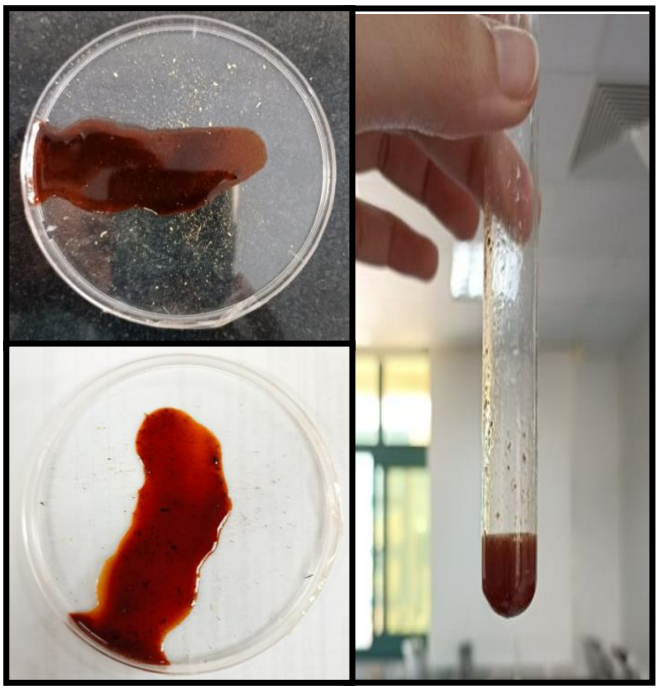

Fig. 3: Qualitative detection of inulin using resorcinol test.

Table 1: Characteristic colours of *S. costus* with different chemicals in visible light.

Reagent	Characteristic colour in visible light (after 2 hrs.)
Powder as such (untreated powder)	Dark brown or yellowish brown
Powder + Water	Dark brown
Powder + conc. HCl	Deep violet
Powder + dil. HCl	yellowish brown
Powder + conc. H_2SO_4	Black
Powder + dil. H_2SO_4	cherry red
Powder + 5% KOH	Brown
Powder + 32% NH_4OH	Brownish green
Powder + Iodine N/50	Yellowish brown
Powder + Diethyl ether	Light brown
Powder + Chloroform	Bright orange brown
Powder + Methanol	Orange brown
conc.- Concentrated, dil.- Diluted, hrs.- Hours	

Table 2: Summary of fluorescence studies for *S. costus* root powder.

Reagent	Under UV light (after 2 hrs.)
Powder as such (untreated powder)	Ivory
Powder + Water	Teal blue
Powder + 5% KOH	Brown
Powder + 32% NH_4OH	Brownish green
Powder + 50% Ethanol	Mint green
UV- Ultra violet, hrs.- Hours	

Table 3: Determination of some quality control parameters of dried roots of *S. costus*.

Quality control parameter	Root of *S. costus* %w/w
Extractive values	
Hot extraction	
Water soluble extractive	24.7 %
Methanol soluble extractive	20.803%
Cold extraction	
Water soluble extractive	21.6 %
Methanol soluble extractive	19.82%
Chloroform soluble extractive	3.0%
Diethylether soluble extractive	1.857 %
Foaming index	Nil.

Preliminary phytochemical screening of *Saussurea costus* (Falc.) Lipsch.

The majority of the pharmacological properties that crude drugs exhibit are related to the presence of active constituents. A study of the root's phytochemistry found the presence of variety of phytoconstituents such as terpenoids, flavonoids, steroids, tannins, and glycosides. The results of phytochemical screening were summarized in table 4.

Table 4: Preliminary phytochemical screening of *Saussurea costus* roots.

	Phytoconstituent	Result
1	Terpenoids	+
2	Anthraquinone	+
3	Lignans	+
4	Flavonoides	+
5	Alkaloids	+
6	Glycosides	+
7	Steroids	+
8	Tannins	+
9	Cardiac Glycosides	+
10	Quinones	+
11	Resins	+
12	Phenol/Polyphenols	+
13	Coumarins	+
14	Carboxylic acid	-
15	Lipids	-
16	Emodins	-
17	Phlobatannins	-
18	Saponins	-
19	Leucoanthocyanins	-
20	Volatile oil	-
+ : Presence, - : Absence		

Conclusion

Some pharmacognostical parameters, such as macroscopical and microscopical characteristics, drug fluorescence analysis, different extractive values, as well as preliminary phytochemical screening of the plant root, were studied in this study. These pharmacognostic investigations of *S. costus* root may be valuable in supplementing information on its identification parameters. In conclusion, the current pharmacognostical assessment of *S. costus* root can be successfully used in laboratory and manufactory operations for the identification of plant powder and detection of adulteration, which are essential factors for quality, efficacy, and safety of herbal drugs.

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Conflict of interests

Authors do not have any conflict of interests.

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دراسات عقاقيرية لجذور نبات السوسوريا كاستس فالك ليبش

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النبات الذي تم اختياره لهذه الدراسة هو نبات السوسوريا كاستس فالك ليبش ، الذي ينتمي إلى العائلة النجمية وتُستخدم جذوره تقليديًا لعلاج مجموعة متنوعة من الحالات ، بما في ذلك عدوى الحلق والزُحار والقرح والربو. كما أظهرت الدراسات أن جذور السوسوريا كاستس لها مجموعة واسعة من التأثيرات العلاجية ، بما في ذلك التأثير المضاد للأكسدة ، والمضاد للأورام ، والواقي للكبد ، والمضاد للالتهابات. في هذه الدراسة ، تم إجراء تقييم عقاقيرى لتوفير معلومات تكميلية حول معايير التعرف على نبات السوسوريا كاستس. يشمل هذا التقييم فحص الصفات المورفولوجية والمجهرية ؛ دراسات التألق الفلوري؛ تحديد المعايير الفيزيائية الكيميائية؛ والفحص الكيميائي النباتي. كما تم إجراء الكشف الكيميائي النوعي للأنيولين في مسحوق الجذر. ولقد وجدت الدراسة المجهرية وجود قنوات الراتينج وأوعية نسيج الخشب وبلورات الإنيولين وخلايا فلينية وقصيبات هوائية والياف. وقد تم عرض صور العناصر المجهرية في هذه الدراسة. كشفَ الفحص الكيميائي النباتي الأولي لجذر السوسوريا كاستس عن وجود مركبات terpenoids و flavonoids و alkaloids و tannins والعديد من المكونات الأخرى. ويمكن استخدام التقييم العقاقيرى الحالي لجذور السوسوريا كاستس بنجاح في العمليات المعملية والمصنعية لتحديد مسحوق النبات والكشف عن الغش ، والتي تعتبر من المعايير الهامة للنبات قبل استخدامه.