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Review Article

In vitro Establishment of *Saussurea costus* - An Endangered Medicinal Herb

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ABSTRACT

S. costus is a medical plant naturally found at an altitude of 2000 to 3500 m above sea level in the northern Himalayan region. It is one of the 62 species of medical plants present in the Indian Himalayan Region (IHR). It has various phytochemicals as its constituents; some dominantly present phytochemicals in *S. costus* are anthraquinones, costunolide, dihydrocostus lactone, etc. *S. costus* is not only used in the Indian medical system but is also a part of Chinese and Tibetan medical systems it has shown various therapeutic properties such as anti-cancerous activity, anti-ulcerogenic activity, anti-inflammatory activity, etc. It is also used as a component of perfume formulation due to its distinctive aroma. *S. costus* has been declared as endangered and critically endangered by the International Union for Conservation of Nature and Natural Resources (IUCN) and the Red Data Book (RDB) of India respectively. Several factors contribute to its endangered status including environmental change, over-exploitation, etc. Thus, it becomes crucial to establish a procedure for the *in vitro* establishment of *S. costus* because the indigenous methods are not significantly useful due to the long cultivation cycle needed for crop harvesting and also due to the low germination percentage and less seed availability of the plant.

Keywords: *S. costus*, Medical plant, Phytochemicals, *In vitro* establishment, Medical usages, Indigenous usages.

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INTRODUCTION

Taxonomy

The taxonomic classification of *S. costus* is as follows.

Kingdom	Plantae
Subkingdom	Virideplantae
Infrakingdom	Streptophyte
Division	Tracheophyta
Subdivision	Spermatophytina
Infradivision	Angiospermae
Class	Magnoliopsida
Superorder	Asteranae
Order	Asterales
Family	Asteraceae
Genus	*Saussurea*
Species	*costus*

Morphology

S. costus is a lean, evergreen plant and can be as long as 2 m. The feature of the plant also includes the presence of certain fibers whereas the root of the plant can grow up to 60 cm long and emits a unique aroma; It possesses concave, bent, flat, and hairy fruits; organized in terminal and apical clusters (M. M. Pandey *et al.*, 2007). Plant flowers are navy in color have membranes, are clustered, and possess uneven bumps; upper leaves are small while leaves at the base are larger and have lobes along with a stretched-out winged stack (Zahara *et al.*, 2014).

Origin

Around 62 species of *S. costus* are indigenous to the Indian Himalayan Region and hold significant medical importance (Rathore *et al.*, 2021). *Saussurea costus* (synonym *Saussurea costus* lipsch) is found at an altitude of 3000-3500 m higher than the sea level in the region of the Northwestern Himalayas. It is also finely distributed in certain states of India such as Kashmir, Uttarakhand, etc., (Gwari *et al.*, 2013) as well as in certain regions such as Suru Valley in Ladakh, Kishenganga, and the higher altitude regions such as the Chenab valleys in Jammu and Kashmir (Kala *et al.*, 2006).

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Phytochemicals present in *S. costus*

Phytochemicals and types of phytochemicals extracted from the S. costus

Phytochemicals are the primary or secondary metabolites that the plant produces, they are not just important for the better development of the plant but also protect it from harmful agents (Martinez *et al.*, 2017). Phytochemicals are proven to help in treating various human illnesses. Phytochemicals are reported to demonstrate chemoprevention and chemotherapeutic activities in cancer cell lines and animal models (Bathaie *et al.*, 2015).

The roots of *S. costus* contain various phytochemicals such as monoterpenoids, dehydrocostus lactone, flavonoids, glycosides, triterpenes, etc. (Rathore *et al.*, 2021). It also contains alkaloids, anthraquinones, costunolide, dihydrocostus lactone, etc. (Zahara *et al.*, 2014). The roots of the *S. costus* are particularly abundant in the sesquiterpenoid (Rathore *et al.*, 2021). Sesquiterpenoids are present in a larger proportion (79.80%) of the essential oil derived from roots than monoterpenoids (13.25%), Dehydrocostus lactone is also a significant component of the *S. costus* (Liu *et al.*, 2012).

Dehydrocostus lactones and costunolide have shown prevention against breast cancer (Peng *et al.*, 2017) and they are also proven to be effective against a variety of microbiological diseases. Dehydrocostus lactone can treat the disintegration of cells, growth restriction, and antibacterial attributes (Pandey *et al.*, 2007);(Peng *et al.*, 2017). Dehydrocostuslactone has an impact on apoptosis and its mechanism of action in human leukemia cells (Chang and Kim, 2008). Potential anti-cancer drugs contain various phytochemicals such as costunolide, and dehydrocostus lactone as their constituent (Chen *et al.*, 2008; Cho *et al.*, 2004; Jeong *et al.*, 2002). Several reports point out the idea that the herb-*S. costus* has anti-parasitic, anti-diabetic, CNS depressive, and hypolipidemic qualities (Rathore *et al.*, 2021).

Usage of the *S. costus*

Indigenous uses

The shoot system of the *S. costus* acts as both lubricant and feed in the district of Himachal Pradesh (Butola and Samant, 2010). Its roots are used as perfume and as a pesticide in the Indian Himalayan region to protect wool clothing from insects (Rathore *et al.*, 2021), along with that it is also been used in antiseptic, hair oils, high-quality perfume formulations and locally it used for curing stomachaches, colds, tooth pain, ulcers, etc. (Rathore *et al.*, 2021). *S. costus* has been recommended for noncyclic menstruation and lower body pain (S. G. Ko *et al.*, 2005).

Medical usage

Anti-cancerous activity

The extracts from the *S. costus*, such as costunolide have the potential to develop into potent drugs for cancer treatment as there are numerous of evidence that points out their anticancerous effect (Rasul *et al.*, 2012). Dihydrocostus lactone present in its roots has shown a significant effect in the

Methods used in the extraction of phytochemical

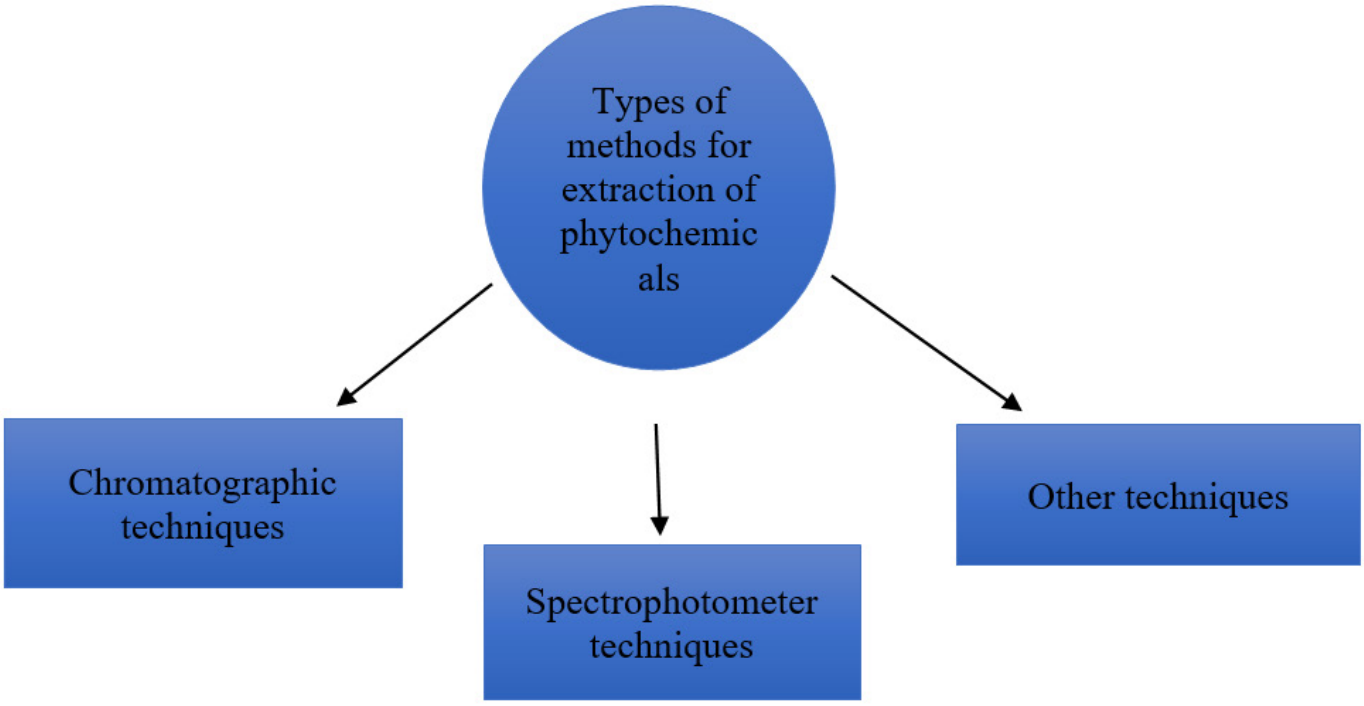


Figure 1: Flowchart representing different techniques in which the phytochemicals of *S. costus* can be extracted.

Table 1: List of different types of methods as well as the techniques for extraction of phytochemicals from *S. costus* (Rathore *et al.*, 2021).

Extraction type	Technique involved
Chromatographic techniques	**HPLC:** High-Pressure Liquid Chromatography. **TLC:** Thin Layer Chromatography. **LC:** Liquid Chromatography **HPTLC:** High Pressure Thin Layer Chromatography. **MPLC:** Medium Pressure Liquid Chromatography. **LPLC:** Low-Pressure Liquid Chromatography.
Spectrometric techniques/ apparatus	**IR spectrophotometer:** InfraRed spectrophotometer **NMR:** Nuclear Magnetic Resonance. **MS:** Mass Spectrometry analysis **HR-QTOF-MS:** High Resolution-Quadrupolar Time of Flight-Mass Spectrometry.
Other techniques	Hydrodistillation Soxhlet Extraction

suppression of the development of cancer cells (Lin *et al.*, 2015). The phytochemicals extracted from the *S. costus* leaves showed impressive anticancerous effects on the cancer cell lines, these phytochemicals arrest the cell cycle in the G1 phase, halting the DNA replication, and inducing apoptosis (Rathore *et al.*, 2021). *S. costus* extracts activate the mitochondrial pathway which increases the level of pro-apoptotic proteins decreases the level of antiapoptotic protein expression level and induces apoptosis (Shati *et al.*, 2020).

Various reports suggest the phytochemicals extracted from *S. costus* such as costunolide, dehydrocostuslactone, and costic acid are effective in the treatment of numerous types of cancers such as lung cancer, prostate cancer, oral cancer as well as gastric cancer (Hung *et al.*, 2010), (Kim *et al.*, 2008; Tian *et al.*, 2017), (Moon *et al.*, 2013), (S. G. Ko *et al.*, 2004). When a human cell line was exposed to certain phytochemicals such as costunolide, dehydrocostuslactone, etc. tumor necrosis factor-alpha and interferon-gamma were activated, Alantolactone as well as caryophyllene were employed as treatments for the human keratinocyte cell line HaCaT (Rathore *et al.*, 2021). Costus causes fluctuations in the tumor suppressor genes and helps in restraining the growth of human breast, and liver cancer cells, etc. (Shati *et al.*, 2020). The cytotoxic effect of chloroformic extracts from *S. costus* was studied on various cancer cell lines such as lung cancer, Breast cancer, and colon cancer, it has been seen that effect of the extracts of the plant had a similar effect as that of the standard drugs such as doxorubicin provided to the patients suffering from breast cancer while no significant effect of the drug was observed on other cell line (Sunkara *et al.*, 2010). Shikokiols, an extract of *S. costus* has also shown anticancerous activities on various types of cancer cells as it arrests the cell cycle of the cancerous cells at the G2 phase and then induces apoptosis (S. G. Ko *et al.*, 2005).The sesquiterpene lactones are observed to possess anticancerous activity against prostate tumors (Tian *et al.*, 2017). KB cells number was observed to be reduced when treated with IC_{50} value (300 µg/mL) of *S. costus* extract as it reduces the growth of the cancer cells (Moon *et al.*, 2013).

Anti-ulcerogenic activity

UL409 is an herbal formulation that contains *S. costus* extracts and is observed to possess anti-ulcer activity as it increases mucus discharge and improves gastric cell line protection (Gautam and Asrani, 2018). It has been reported (Sutar *et al.*, 2011) that *S. costus* root extract when used in a particular dosage reduces the build-up of gastric acid and total acid. Still, it has also been noted that the effect was lower as compared to a standard drug.

Immunomodulatory activity

Higher doses of *S. costus* extract suggested the possibility of humoral and cellular immunomodulatory activity in the immune system (R. S. Pandey, 2012). Costunolide, an extract of *S. costus* is observed to increase the levels of tyrosine phosphorylation in response to T-cell receptor cross-linking, according to research on the drug and dehydrocostus lactone (M. M. Pandey *et al.*, 2007; Taniguchi *et al.*, 1995). KM1608 is an herbal formulation that contains the extracts of *S. costus* and is observed to induce immune-stimulatory effects in murine (Trinh *et al.*, 2020).

Antidiarrheal activity

According to the report submitted by (Negi *et al.*, 2013), it has been observed that the costus oil can help in controlling diarrhea. It has been observed that when a group of Wistar rats induced with diarrhea were treated with castor oil, there was 26% to 67% inhibition in the diarrhea. There are a number of reports that claim the usage of costus for diarrhea and thus enhances its anti-diarrheal activity.

Larvicidal activity

Costus is proven to be a safer and more organic option as a larvicide performed against *A. albopictus* (Rathore *et al.*, 2021) as it has been observed by (Liu *et al.*, 2012), that certain extracts from the costus such as steroids, dehydrocostus lactone, and essential oil hinder the growth of the larvae of various insects, especially against *A. albopictus*, which transmits various diseases such as *dengue* fever. The larvicidal capacity of *S. costus* was confirmed by exposing the larvae to the methanolic root extracts of *S. costus* for 24 hr. and showed the best larvicidal activity with LC_{50} and 90 values (Ali and Venkatesalu, 2020).

Other uses of Costus

S. costus contains yellow -brownish oil extracted from its roots and it contains 39 components including dehydrocostus lactone and beta-costus as its significant constituents (Zahara *et al*., 2014) hold significant value not just in the medical but other industrial sectors too. The *S. costus* oil has several benefits such as it can be formulated in hair oil, expensive perfumes, insect repellents, incense as it can blend with several other components such as vetiver, patchouli, rose, violet, and sandalwood (Rathore *et al*., 2021). The costus oil composition depends upon the differences in the environmental condition, phenophase, ecotype, and chemotype as well as the genetics of the plant also influence the secondary metabolites of the plant (Rathore *et al*., 2021). Other bioactive components present in the *S. costus* roots include sesquiterpenoids lignans, triterpenes, steroids, flavonoids, glycosides, etc.(Benedetto *et al*., 2019).

Perfumery

S. costus root oil contains sesquiterpene lactones in large concentrations which is responsible for various allergic contact dermatitis cases but still it holds high demand in perfumery (Gwari *et al*., 2013) because of its nice scent (Butola and Samant, 2010) but the presence of sesquiterpenoid α-methylene lactones, which is a harmful lactone can reduce the usage of *S. costus* root oil in perfumes (Rathore *et al*., 2021).

Biosynthetic pathway of *S. costus*

There are seventeen chromosomes in all in *S. costus* (Singh *et al*., 2018), and despite having high medical importance, the exploration of the plant has not been done intensively, especially at the molecular level but through de novo leaf transcriptome analysis, the biosynthesis pathways of sesquiterpenoid and flavonoid found in *S. costus* were investigated. This helped to shed light on the plant's metabolism and functional genomics (Rathore *et al*., 2021).

Importance of *in vitro* establishment of *Saussurea costus*

Saussurea costus holds a significant value in the industry but due to various factors such as increased consumption, invasion of other species in the environment, deforestation, and some other factors like contamination of crops (Amara *et al*., 2017) as well as heavy exploitation and an unscientific handling of the crop, its number has been significantly reduced, Thus The Red Data Handbook (RDB) of India and the International Union for Conservation of Nature and Natural Resources (IUCN) have declared *S. costus* as endangered and critically endangered, respectively(Siddique *et al*., 2001).

Additional factors contributing to the plant's severely endangered status are the growing demand for it in the pharmaceutical business, the herb's restricted distribution, and overuse. The Council of Scientific and Industrial Research (CSIR) initiated the CSIR Phytopharmaceutical Mission project (HCP 0010) to move the species from the critically endangered to the non-endangered category. This has resulted in the CSIR Institute of Himalayan Bioresource Technology, Palampur, HP, conducting extensive captive cultivation, and producing superior planting material (Rathore *et al*., 2021).

Conventionally the plant is grown through the seeds but this approach is not suitable for the efficient growth of the crop because of lesser seed availability, and low percentage of germination, the cultivation of costus requires a patient approach as it takes up to three years to produce mature roots that are rich in valuable extracts like costus oil and other phytochemicals, This extended cultivation cycle is a significant challenge for plant growers, Therefore, micropropagation or *in vitro* cultivation of the plant becomes an important approach to for mass production of the *S. costus* (Sharma *et al*., 2019).

Micropropagation is the process of vegetative development and multiplication of the plant from plant tissue that are artificially cultivated on nutritional media (Leifert *et al*., 1989). It is one of the main ways that contributes to the industrial cultivation of plants and has a lot of uses. (Jha, 2005). Micropropagation is based on the concept of omnipotence, which is the ability of plant cells and tissues to develop into whole new plants (Bhojwani and Razdan, 1996).

But this technique also includes the risk of somaclonal variance which can be encountered by analyzing the stability of the plant at various level of plant growth. The analysis can be performed at phenotypic, cytological, and phytochemical levels (Ghosh *et al*., 2002). To determine if the regenerated plant is genetically similar to its parent plant, chromosomal tests from tissue culture are necessary (Baran, 2005).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS

HPLC: High-Pressure Liquid Chromatography; **TLC:** Thin Layer Chromatography; **LC:** Liquid Chromatography; **HPTLC:** High-Pressure Thin Layer Chromatography; **MPLC:** Medium Pressure Liquid Chromatography; **LPLC:** Low-Pressure Liquid Chromatography; **IR spectrophotometer:** InfraRed spectrophotometer; **NMR:** Nuclear Magnetic Resonance; **MS:**

Mass Spectrometry analysis; **HR-QTOF-MS:** High Resolution-Quadrupolar Time of Flight-Mass Spectrometry; **HaCaT:** High sensitivity of human epidermal keratinocytes; **CSIR:** Council of Scientific and Industrial Research; **RDB:** The Red Data Handbook; **IUCN:** International Union for Conservation of Nature and Natural Resources.

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