

A Critical Review on Phytochemical Analysis of *Smilax China Rhizomes*

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ABSTRACT

The climbing herb *Smilax china* L. grows in humid, temperate climates all around the world. When investigating the whole therapeutic potential of this medicinal plant, regional diversity should be taken into account, as evidenced by the reported variations in TPC, TFC, and chemical makeup. More research should be done to find the genetic and environmental elements that cause these regional variances, such as soil composition, climate, and altitude, as well as the conditions that encourage the production of desired plant chemicals. Chemical testing and GC-MS analysis qualitatively revealed the polar and nonpolar components present in the methanolic extracts of both *Smilax china*. The rhizome of *S. china* was used to isolate quercetin. In the concentration range of 0.1–0.8 µg/ml, chemicals TA and TAR showed excellent linearity. During the chemicals' retention period in the sample solution, no interference was found (TA and TAR). The present validated approach has an average recovery rate between 97% and 102%. For the purpose of measuring the components TA and TAR in *S. china* extracts and other dietary supplements, a rapid reverse-phase HPLC method was created and verified. This method is timely, exact, and accurate.

Keywords: TFC, TPC, GC-MS, HPLC, HPTLC

INTRODUCTION

Many ailments can be cured by medicinal plants that are enhanced with various bioactive substances¹. The roots, rhizomes, stems, barks, leaves, aerial portions, and whole parts of medicinal plants are all home to oligosaccharides and their esters, an important class of phytochemical substances. They are essential in the treatment of illnesses in addition to being the components that store energy. The isolation and structural elucidation of oligosaccharides and their esters from Chinese medicinal plants had been the subject of several reviews and reports prior to 2003; however, few biological activities, such as protein kinase C inhibitory and cancer chemopreventive properties, had been documented. Over the past few decades, a greater number of oligosaccharides and their esters have been found from traditional Chinese medicines thanks to advancements in isolation and identification procedures². The rhizomes are tonic, febrifuge, laxative, depurative, thermogenic, anodyne, anti-inflammatory, and bitter. It is used to treat helminthiasis, colic, constipation, gas, and

dyspepsia. It helps with psoriasis, leprosy, and other skin conditions. It is used to treat neuralgia, fever, epilepsy, and insanity. Syphilis, strangury, seminal weakness, and general debility are among its uses. eliminates microorganisms, purifies blood, facilitates absorption, and detoxifies organs. Additionally, it enhances sweating, protects the liver, increases urine, and improves digestion³. The substances that plants produce as a result of regular metabolic processes are known as phytochemicals. Phytochemical composition is typically complicated and varies depending on the plant's origin and developmental stage. These secondary metabolic products are linked to a variety of plant functions, some of which include therapeutic effects⁴. *Smilacis Glabrae Rhizoma* (SGR) is defined in the 2020 edition of the Chinese Pharmacopoeia as the dried rhizome of *Smilax glabra* Roxb., a member of the Liliaceae family that is mostly found in Guangdong, Hunan, and Zhejiang. Historical writings, such as the Compendium of Materia Medica, make a distinction between the red and white forms of SGR and give the latter a greater medicinal value.

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SGR is said to boost musculoskeletal health, support stomach and spleen function, and accelerate metabolic processes that result in better circulation and relaxed muscles. It has long been used to treat skin diseases, particularly malignant sores and carbuncles, as well as to relieve pain and gastrointestinal issues. Flavonoids, steroids, saccharides, phenolic glycosides, volatiles, organic acids, and phenylpropanoids make up SGR's pharmacological profile⁵. *Smilax china* L., also referred to as "Jin Gang Teng" or "Ba Qia", is a common traditional Chinese medicine (TCM) used to treat rheumatic, arthritic, and diuretic conditions, detoxication, lumbago, gout, tumours, and inflammatory diseases. In some parts of China, it is also consumed as food. The dried rhizome of *Smilax china* L., a member of the Smilacaceae family, is called "chobchini" in Hindi. It has anti-inflammatory, diuretic, anti-diabetic, anti-psoriatic, and digestive qualities and includes fat, sugar, glycoside, colouring matter, saponin, gum, tannin, cinchonin, smilacin, and starch⁶. Several saponins, including smilaxin, prosapogenin A of dioscin, gracillin, dioscin, pseudoprotodioscin, methylgracillin, and methylprotodioscin, were isolated from this plant as a result of earlier phytochemical investigations⁷. Traditional Chinese medicine (TCM) uses it to treat a number of illnesses, particularly syphilis, acute bacillary dysentery, chronic nephritis, and chronic pelvic inflammation. It is also used locally in Pakistan's northern regions to treat inflammatory conditions like asthma and rheumatism⁸. In China, it has been widely utilised for anticancer, acute bacillary dysentery, chronic nephritis, and syphilis clinical therapy. In mice and rats, the *Smilax china* L. rhizome complex (SCR) has an effect on a variety of tumours⁹. "Sarsaparilla" is a popular name for the *Smilax* species. They have been used in folk medicine as a rheumatism tonic since the 16th century. Furthermore, *Smilax zeylanica* has been used as a carminative, in dropsy (plant), to relieve burning sensations in the feet associated with vesicular watery eruptions, and for venereal diseases, antifertility, analgesia, and anthelmintic action (leaf)¹⁰. There are over 300 species of climbing shrubs in the genus *Smilax* (Liliaceae family). The genus's plants are found in warm regions of East Asia and North America, as well as in tropical regions of the world¹¹. Parkinson's disease is a neurological degenerative

condition that frequently manifests as stiffness, slowness, and tremors in movement. Additionally, the substantia nigra and other brain regions are affected, leading to gradual neurodegeneration. As the illness progresses, non-motor symptoms, including dementia and dysautonomia, frequently manifest. Parkinson's disease is caused by a decrease in mitochondrial complex-1, a number of cell-damaging processes, such as protein aggregation, apoptosis, calcium homeostasis, inflammation, excitotoxicity, abnormal energy metabolism, and strong links between genetics and the environment¹². According to Ayurvedic, Siddha, and Unani medicine, the rhizomes of *Smilax china* Linn. (Liliaceae) have been used to treat gout, rheumatism, epilepsy, syphilis, colic, skin conditions, chronic nerve illnesses, neuralgia, and insanity¹³. In Ayurvedic and traditional Chinese medicine, *Smilax china* (SC), sometimes referred to as China root and Chob cheeni, is a significant and well-liked herbal therapy that has been used to treat a variety of illnesses. Steroid saponins and flavonoid glycosides are abundant in the genus *Smilax*. SC includes alkaloids, phenolic acids including caffeic acid, gentisic acid, and trans-o-coumaric acid, as well as flavonoids like quercetin, resveratrol, oxyresveratrol, astaxin, rutin, and engeletin, and steroidal saponins like dioscin, according to preliminary phytochemical screening¹⁴. Natural products, such as steroidal compounds, are increasingly being used as lead compounds in drug discovery techniques as well as therapeutically active molecules. For instance, a few years ago, it was shown that several steroidal saponins and sapogenins have intriguing anticancer effects with a generally safe usage profile. Among these substances, diosgenin is a well-known steroidal sapogenin that was created by hydrolysing the saponin dioscin. It is derived from a number of plants, including species of *Dioscorea*, *Trigonella*, *Costus*, and *Smilax*, and has long been used in traditional medicine to treat a wide range of illnesses. Over the years, numerous academics from around the world have been interested in this steroid due to its significant industrial value¹⁵. Globally, cancer is the leading cause of mortality from a non-communicable disease. Liver cancer is one of the leading causes of cancer-related deaths worldwide. Its prevalence rate has steadily increased over the past ten years due to a number of factors, including alcohol consumption, nonalcoholic fatty liver disease, and the increased



prevalence of hepatitis B or C infections¹⁶. At the moment, novel strategies are being used to stop the development and spread of human health issues. One tactic is the use of substances from traditional medicinal herbs, edible dietary plants, and seeds that can be used either alone or in conjunction with current therapeutic methods to prevent and treat cancer. A wide range of human ailments, such as obesity, neurological disorders, cancer, metabolic syndromes, cardiovascular disease, and diabetes, have been shown to be effectively treated and prevented by natural medicines made from medicinal herbs, various vegetables, plants, and fruits that have promising health benefits¹⁷. The liver carries out several essential functions, including metabolism, secretion, and storage, and it is essential in controlling physiological processes. Additionally, the liver is in charge of detoxifying medications and xenobiotics. Therefore, major health problems may arise from the development of liver illnesses. These illnesses fall into one of three categories: non-inflammatory diseases (hepatosis), acute or chronic liver inflammatory diseases (hepatitis), or degenerative disorders (cirrhosis). Infections, autoimmune diseases, excessive alcohol use, and the ingestion of harmful substances, including peroxidised oil, medications, antibiotics, chlorinated hydrocarbons, and carbon tetrachloride, can all directly cause liver ailments. All civilisations are impacted by the widespread issue of alcoholism and abuse. One of the leading causes of chronic liver damage brought on by excessive alcohol use that kills people all over the world is alcoholic liver disease (ALD)¹⁸. Plants have been utilised extensively for food, building materials, clothes, spices, colours, and medicine from the beginning of time. Traditional medical practitioners in many societies use plants, either alone or in specific combinations, for primary treatment. Many European, American, and African nations, including China, India, Bangladesh, Pakistan, Bhutan, and Thailand, use a variety of plant species as medicine to treat a wide range of illnesses. Due to the high expense of contemporary pharmaceuticals and the lack of access to them in distant areas, plant-based treatments are popular in developing and underdeveloped nations. Additionally, plants have been an essential part of traditional medical systems such as Siddha, Ayurveda, Unani, and traditional Chinese medicine (TCM)¹⁹. Because traditional medical herbs have low

toxicity and relatively high potency, natural ingredients have been popular for decades for creating new cosmetics. We recently discovered that *Smilax china* L. root extract successfully inhibited *C. acnes* development. Numerous secondary metabolites have been identified from *Smilax china*, a medicinal plant. For example, *Smilax china* was used to isolate cytotoxic phenylpropanoid glycosides, steroidal saponins, and stilbenes and flavonoids such as oxyresveratrol, resveratrol, and scirpusin A. Smilasides are the novel chemicals, phenylpropanoid glycosides, that were extracted from *Smilax china* stems²⁰. Previous chemical and biological studies have revealed that SCR includes flavonoids, tannins, and saponins and has intriguing anti-inflammatory and anticancer effects²¹. With a long history of clinical usage in the treatment of hyperuricemia *Smilax glabra* Roxb (SGR) is a traditional Chinese medicine renowned for its therapeutic and edible qualities²². Metabolic diseases, including obesity, diabetes mellitus, dyslipidaemia, and hypertension, are on the rise globally. Numerous important molecules are involved in the pathophysiology of these illnesses, which have a complex origin²³. The herb known as chobchini (*Smilax china* Linn) has a long history. Since it is frequently grown in China and is thought to be disease-free, the genus name "Smilax" is derived from the name of a climbing shrub that is widely distributed and has hooks and tendrils. Aphrodisiac, antiepileptic, anti-inflammatory, nervine tonic, anti-rheumatism, anti-syphilitic, demulcent, and other conditions are treated with leaves, which are used as tea and in other forms to detoxify the body. Additionally, the herb is thought to be a powerful stimulant and resolvent for constipation, dyspepsia, and flatulence. beneficial for neuralgia and skin conditions. It is primarily used to treat rheumatoid arthritis and gout. Additionally, it fortifies the entire body, and the nervous system is advantageous in cases of fever. The high quantity of flavonoids, smilacin, tannin, resin, cinchonin, and saponin in *Smilax* is responsible for many of its beneficial effects²⁴. Diabetes is an endocrine system metabolic disease. It is regarded as one of the most dangerous endocrine disorders. The illness affects people all over the world, and in most regions of the world, its prevalence is rising quickly. People with diabetes have high blood glucose levels because their bodies are unable to manufacture or use insulin as they should.



Diabetes's high prevalence, morbidity, and mortality are making it the third leading cause of death worldwide, behind cardiovascular disease and cancer²⁵. Numerous phytochemicals that have been extracted from *S. glabra* roots have a variety of bioactivities; for instance, resveratrol, eurythrin, engeletin, aspirin, and smilbin have liver-protective and antioxidant properties. When rats developed adjuvant-induced arthritis, the rhizome, an aqueous extract of *S. glabra*, exhibited immunomodulatory action²⁶. Since there was no word for "cancer" or "tumour" in ancient China, a patient with cancer would be diagnosed with "Qi" deficiency, blood stasis, or slippery pulse. These outdated diagnoses might have modern equivalents in pathogenic gene mutations or aberrant cytokine signalling²⁷. From prehistoric times to the present, plants have been utilised as useful sources of a variety of products. From one generation to the next, people have been taught how to employ plants and their unique qualities for human health, cosmetics, and illness treatment. Rather than undergoing technical processing such as extraction, modification, blending, or purification, plants were used directly in their early applications. Thus, plants were either eaten raw or were only boiled, dried, or crushed²⁸. In addition to being a rich source of starch, it is a component of winemaking. Its leaves and shoots are frequently eaten as vegetables in China, while in Korea, the leaves are added to rice cakes to improve their flavour²⁹. This Liliaceous plant's dry root is used to make *Smilax china* L. extract. *S. china* root extract possesses antibacterial, anti-inflammatory, antioxidative, anti-cancer, and other pharmacological properties, according to current pharmacological research³⁰. Globally, exposure to air pollution has been found to be closely linked to higher rates of illness and mortality. Airborne pollutants are known to induce well-established acute and long-term health impacts in humans and to enter the body through a variety of pathways, such as direct ingestion and inhalation as well as skin contact³¹. Because they are the foundation of food webs and chains, plants are essential to all other life on Earth. The study of the interaction between humans and plants is known as ethnobotany. Plants have been used for a variety of reasons since ancient times, including food, fuel, medicine, fodder, building, and transportation. Additionally, plants are used to prepare dyes (colours) and cosmetics as well

as to beautify the body. Traditional medicine relies heavily on plants. Traditional medical professionals use plants alone or in specific combinations to treat veterinary and human illnesses all around the world. People with poor incomes and those who live in remote places without access to contemporary medical care frequently use plant-based medications³². The phytochemical content study of *Smilax china* rhizomes is the main topic of this review article.

MATERIALS AND METHODS

Chang Dae Lee et al. (2024)³³

Sample Extraction: Fresh *S. china* rhizomes that were gathered from various parts of Korea were dried before the roots were extracted from the rhizome samples. After being dried, the rhizomes were chopped into tiny pieces and extracted with 30 mg of pure ethanol (EtOH). A reflux extractor was used to extract the samples in triplicate for three hours using 300 mL of EtOH. To create concentrated EtOH extracts, the extracts were then filtered and concentrated using a rotary vacuum evaporator.

Preparation of Standards and Sample Solutions: Each reference compound (1 mg) and all sample extracts (10 mg) were diluted in 1 millilitre of MeOH. After 20 minutes of sonication, they were filtered through a 0.45 µm polyvinylidene fluoride membrane filter.

Analysis of TPC (total polyphenol content): Initially, 60 µL of each extract was mixed with 40 µL of 2 N Folin-Ciocalteu phenol reagent. After adding the combination to 100 µL of a 7.5% sodium carbonate solution, it was allowed to sit at room temperature for 30 minutes in the dark. A microplate reader was used to measure the absorbance at 760 nm at the conclusion of the experiment. Using a calibration curve as the reference, tannic acid was employed as a standard to quantify TPC.

Analysis of TFC (total flavonoid content): In short, 100 µL of 2% AlCl₃ was mixed with each sample. A microplate reader was used to measure absorbance at 430 nm following a 10-minute incubation period. Using quercetin as the standard, the TFC was computed, and a calibration curve was created.



Abdul Qadir et al. (2020)³⁴

Extraction of plant material: 200 g of plant material (chob chini rhizome) was defatted over the period of 24 hours using 500 mL of n-hexane, shaking occasionally. The drug was filtered and dried before being put into a Soxhlet apparatus for the extraction process. At 55–60°C, 40 g of crude drug and 120 mL of methanol were added to the Soxhlet apparatus, and the drug was continuously extracted for six hours. The same process was used to extract another batch of crude medicine. Following the completion of the extraction, the solvent was lyophilised to create a solid powder (18.7 g MESC-Methanol extract of *Smilax China*), and the solid mass (extract) was subsequently produced by low-pressure evaporation. Following a known protocol, GC-MS was used to examine trace amounts (1 g) of each extract.

Phytochemical screening: To identify the presence of various phytoconstituents, such as alkaloids, phenolics, flavonoids, steroids, reducing sugar, saponins, carbohydrates, gums, glycosides, and tannins, methanolic extracts of *Smilax china* (MESC) and *Salix alba* (MESA) were subjected to an initial phytochemical analysis.

GC-MS analysis of bioactive compounds: GC-MS was used to analyse MESC and MESA. A Shimadzu QP-2010 Ultra with a capillary standard and a nonpolar column 60 M TRX 5-MS (dimension: 30 m, ID: 0.25 mm, film: 0.25 mm) made up the GC-MS apparatus. Helium served as the carrier gas, and the mobile phase flow rate was set at 1.21 mL min⁻¹. The volume per injection was set at 2 µL, and the instrument's oven temperature was increased from 100°C to 260°C at a rate of 10°C min⁻¹. An electron ionisation energy system with 70 eV was employed in GC-MS. It ran for 65 minutes in total. Methanol was used to dissolve the MESC, which was then run entirely within the 10–850 m/z range. The Wiley spectral library search tool was used to evaluate and compare the findings. Within 30 to 35 minutes, the mass spectra were acquired. The comparative percentage of each component was determined by equating its average peak area to the total areas, and other aspects of compound identification, such as the molecular formula, molecular weight, and structure of the bioactive components of test materials, along with their names, were established. By comparing the m/z ratios with samples verified by Sigma-Aldrich and using mass spectra data from the NIST Mass Spectral Library Ver. 2.0 d (2005) and the literature, the specific chemicals were identified.

Vijayalakshmi A et al. (2012)³⁵

Extraction: After being shade-dried and coarsely ground, the *S. china* rhizome was extracted using a Soxhlet apparatus and methanol for eighteen hours. After being filtered and concentrated in a rotary vacuum flash evaporator at a lower pressure, the extracted solution was freeze-dried and utilised for the investigation. **Phytochemical screening:** Using standard protocol, qualitative tests were performed on the entire methanol extract to detect the presence of plant secondary metabolites, including carbohydrates, alkaloids, tannins, flavonoids, saponins, and glycosides.

High-performance liquid chromatography (HPLC) analysis: Using HPLC equipment, the flavonoid content of the sample was quantitatively analysed. (D) A computer-controlled high-pressure-gradient LaChrom-HPLC system (Merck-Hitachi) with an Interface L-7000, two pumps L-7100 (one for each eluent), a diode array detector L-7450, an autosampler L-7200 with a 100 µL sample loop, a solvent degasser L-7612, a high-pressure gradient mixer, and a Rheodyne injection valve 7725i with a 20 µL sample loop was used for the analytical HPLC. LaChrome software version 4.0 was used for device administration, data recording, and analysis. Thermo ODS Hypersil C18 (250 mm × 4.6 mm, 5 µm) in isocratic mode was the column. A mobile phase consisting of acetonitrile, 0.1 M phosphate buffer, and glacial acetic acid (15:85:1, v/v/v) was used to accomplish the separation. Phosphoric acid was used to bring the pH down to 4.0 at a flow rate of 1.0 mL/min. At a wavelength of 300 nm, UV detection was used to track the effluent. Before use, the mobile phase was passed through a 0.45 µm nylon filter. It was determined what percentage of flavonoids was present in *S. china*'s rhizome.

High-performance thin-layer chromatography (HPTLC) analysis with known marker quercetin: For the methanol extract of *S. china* rhizome, a fingerprint profile was created using HPTLC (silica gel G 60F254 TLC plates of E. Merck, layer thickness 0.2 mm). Aluminium-backed plates of 10 cm by 10 cm and covered with silica gel 60F254 (Merck, Mumbai, India) were used for HPTLC. A Camag (Muttentz, Switzerland) Linomat V sample applicator fitted with a 100 µL Hamilton (USA) syringe was used to apply the standard solution of quercetin and sample solution to the plates in bands that were 8.0 mm broad, 30.0 mm apart, and 10.0 mm from the bottom edge of the same chromatographic plate. At room temperature (28±2°C), ascending development was carried out to a distance of 80 mm using toluene,



ethyl acetate, and formic acid (5:4:1) (v/v/v) as the mobile phase in a Camag glass twin-trough chamber that had been saturated with mobile phase vapour for 20 minutes beforehand. Following development, the plates were dried with a hairdryer before being scanned using a deuterium lamp and a Camag TLC Scanner with WINCAT software at 254 nm. Standard quercetin (100 µg/mL) was made as a stock solution in methanol. To achieve quercetin concentrations of 200, 400, 800, 1600, and 3200 ng/spot, various quantities of stock solution, 2, 4, 8, 16, and 32 µL, were spotted onto a TLC plate. It was discovered that the quercetin calibration curve ranged linearly from 200 to 3 200 ng/spot. Peak area data was gathered and shown against the associated concentrations.

Isolation of quercetin: For 24 hours, the *S. china* rhizome was Soxhlet extracted separately in 80% methanol (100 mL/g dry weight) in a water bath. The extract underwent successive concentrations and reconcentrations in petroleum ether (fraction I) at 40–60°C, ethyl ether (fraction II), and ethyl acetate (fraction III). To guarantee thorough extraction in every instance, each procedure was carried out three times. While fraction III demonstrated a positive result in the flavonoid test and underwent TLC, fractions I and II were excluded due to their high fatty component content.

Thin layer chromatography (TLC): One centimetre above the plate's edge, the ethyl acetate fraction (fraction III) and quercetin, the standard reference component, were applied individually. A 200 mL solvent mixture of n-butanol, acetic acid, and water (4:1:5, upper layer) was used to develop these glass plates in an airtight chromatography chamber. A number of additional solvent mixes were also tested, including the forestal system (acetic acid, concentrated hydrochloric acid, water, 10:3:30 v/v/v) and ethyl acetate saturated with acetic acid:water (6:4 v/v). In every sample tested, the solvent mixture of n-butanol, acetic acid, and water (4:1:5, upper layer v/v/v) produced the best results. Following air drying, the produced plates were examined under UV illumination, revealing fluorescent spots in fraction III in every case that matched those of the quercetin reference samples (R_f 0.62). After then, the plates were put in an ammonia vapour-saturated chamber to see how the spots (deep yellow quercetin) looked. For isolated samples, R_f values were computed and compared to the corresponding standard. Refluxing with 7% H₂SO₄ (10 mL/g residue) for five hours hydrolysed fraction III. After filtering the mixture, ethyl acetate was added to the filtrate in a separating funnel for extraction. After being neutralised with distilled water, the ethyl acetate layer was vacuum-

dried. Following the crystallisation of the residue using chloroform, the sample was analysed using IR, mass, and NMR spectroscopy.

Apada Reddy Gangadasu (2022)³⁶

Method of preparation of sample: For five hours, 10 grammes of SC is heated with 100 millilitres of water, methanol, and ethanol separately in a water bath using a reflux condenser. It is then cooled and filtered. The SC-A, SC-Me, and SC-ET extracts are obtained by vacuum-evaporating the filtrate. SC-A, SC-Me, and SC-ET underwent phytochemical screening. Phytosterols are present in SC-A (aqueous extract of *Smilax china*). Proteins, amino acids, and saponins are all present in SC-Me (Methanol Extract of *Smilax China*). Protein, amino acids, phytosterols, glycosides, phenolic compounds, flavonoids, and alkaloids are all present in SC-ET (ethanol extract of *Smilax China*).

Test for flavonoids:Compound 1 (SC Me 1): Quercetin and flavonoids are clearly visible. The sample turns yellow when dissolved in sodium hydroxide, and the colour vanishes when diluted HCl is added.

HPLC Analysis: The component extracted from the methanolic extract of *Smilax china* was found to have a retention time of RT 4.06, whereas the pure quercetin standard was found to have a retention time of RT 4.06. These results confirm quercetin's presence in *Smilax china* rhizomes under similar circumstances. **UV Analysis:** The absorbance maximum of the crude extract is 257 nm. The absorption maximum of SC ME-I is 262 nm. The absorption maximum of SC ME II is 272 nm.

Cheng Zhong et al. (2017)³⁷

Isolation and Identification of Compounds: The ethanol extract of *S. china* rhizomes was separated using ethyl acetate and then subjected to column chromatography using MCI gel CHP 20P, Sephadex LH-20, Toyopearl HW 40F, silica gel, and preparative HPLC in order to develop a new triflavanoid along with 19 recognised compounds.

Yun Ma et al. (2023)³⁸

Preparation of *Smilax china* L. Extracts: Initially, 250 g of *S. china* were extracted in triplicate using five times the volume of 95% ethanol for one hour each. After adding the filtration, the mixture was concentrated in order to eliminate the alcohol. An AB-



8 (500 mL) macroporous resin was then used to filter the mixture after 1250 mL of water containing 0.2 g/mL raw pharmaceutical ingredients was added. Eight times the volume of 40% ethanol was used for elution, after which the liquid was concentrated and 1000 millilitres of water were added. Using a filter column with polyamide mesh (80–100 mesh, 140 g), the suction filtrate was eluted with twice the volume of 20% ethanol. 0.9 g of extract was obtained by freeze-drying the samples after they had been concentrated at lower pressure.

Determination of Flavonoid concentration: UPLC was used to measure the extract's flavonoid concentration. The extract that was produced was called flavonoids from *S. china* rhizome (FSCR).

Qing-Feng Zhang (2013)³⁹

HPLC Analysis: A Waters 600 system was used to conduct the HPLC analyses. A 250 mm x 4.6 mm i.d., 5 mm Agilent Zorbax SB C18 column was utilised. Using a linear gradient program of 15–20% (A) in 0–15 min and 20–40% (A) in 15–40 min with a flow rate of 1 mL/min, the mobile phase was composed of acetonitrile (A) and 0.1% acetic acid aqueous solution (B). The chromatograms were displayed at 291 nm, while the DAD spectral data was acquired between 200 and 400 nm. 10 mL was the injection volume for the sample.

HPLC–MS/MS: Examination It was an Agilent 1100 system with a quaternary pump and a vacuum degasser. Mass analysis and detection were performed using an AB MDS Sciex (Concord, Ontario, Canada) API 2000 triple-quadrupole mass spectrometer fitted with a Turbo Ion Spray ESI source. AB MDS Sciex's Analyst 1.1 software was used to acquire the data. The column, flow rate, solvent gradients, injection volumes, and other experimental parameters were identical to those previously mentioned. After splitting, only roughly 35% of the eluent was added to the ESI source. The negative ion mode was used to run the mass spectrometer. As a nebuliser, curtain, and collision-activated dissociation gas, ultrapure nitrogen was employed at 20, 10, and 1 (instrument units), respectively. At 4500 V and 400°C, respectively, the ideal Turbo Ion Spray voltage and temperature were established.

Preparation of Samples: A 40-mesh sieve was used to filter and finely homogenise the dried RSC. After precisely weighing 0.5 g of the material, 25.0 mL of 40% ethanol was used to sonicate it for 20 minutes. The combination was utilised as the sample solution

for HPLC analysis after being adjusted to its initial weight and filtered through a 0.22 mm pore size filter.

Quantitative Method Development: Stock solutions containing 1.0 mg/mL of chlorogenic acid, aspirin, engeletin, taxifolin, and resveratrol were made in 50% methanol and kept at 4°C, accordingly. The stock solutions were diluted with 50% methanol to create new, lower-concentration mixed standard solutions. Using linear regression of the mean peak area vs concentration, the calibration curve for each drug was carried out in triplicate with six distinct concentrations. The method's repeatability was assessed by measuring intra- and inter-day variability. Six injections of S01 were made on a single day to test the intraday repeatability, and five separate days were used to assess the interday repeatability. Three distinct concentration levels of mixture standard solutions were added to S01, and triplicate analyses were performed at each level to assess the accuracy of the devised procedure. The mean recoveries of the five chemicals from the spiked standard solutions were calculated in order to assess the accuracy.

Subashini Subramanian et al. (2022)⁴⁰

Isolation: The hydroalcoholic extract (50 g) was column chromatographed (60-120 mesh) on silica gel. Eluted using different eutectic mixtures, such as the ratios of hexane to ethyl acetate. The same fractions were combined and evaporated using a rota-vacuum. The fractions collected during the eluting process with hexane: ethyl acetate (80:20) yielded compound 1 (yield 56 mg), while the fractions collected during the eluting process with hexane: ethyl acetate (65:35) yielded compound 2. (Produce 135 mg). Using a hexane:ethyl acetate (6:4, v/v) mobile phase, these compounds produced a single spot on TLC at R_f 0.41 and 0.83. The spectral data was analysed using NMR and compared to taxifolin and taxifolin 3-O-rhamnoside.

Instrumentation: An LC-20AP was used in HPLC investigations. The LC-20AP system had a column oven, degassing unit, low-pressure gradient unit, and isocratic unit. An RP-C18 column with a 20- μ L aliquot sample loop capacity, a Shimadzu SPD-M20A photodiode-array detector with an isothermal flow cell, a low-pressure quaternary mixing pump, and a 20 μ L Hamilton injector were used in this experiment. Data was gathered and processed by the HPLC apparatus using a lab solution. A Shimadzu spectrometer was used to obtain the UV spectra.

Selection of Wavelength: Methanol was used to make a standard solution of 1 mg per millilitre. The



UV spectra were obtained by scanning the standard solution between 190 and 800 nm after the RP-C18 column was injected with 20 µg/ml solutions using the Hamilton injector. A PDA detector was used to measure the eluents' maximal absorbance by TA and TAR at 254 nm.

Preparation of standard and sample solutions: Ten milligrammes of the *S. china* sample were dissolved in three distinct solvents—ethanol, methanol, and an alcohol-based hydro-alcoholic solution—in order to create study samples. In volumetric flasks, 7 mg of chemicals were dissolved in 7 ml of methanol (1 mg/ml) to create the standards TA and TAR. Following a thorough mixing process, the mixture was filtered through Whatman filter paper and then again through a 0.2 µm membrane filter. The volume was up to the methanol threshold in order to reach the final concentration. In methanol, the dilution series of 0.1, 0.2, 0.4, 0.6, and 0.8 µg/ml were created and used as destination concentrations at 100% from the previously mentioned standard stock solution.

Assay of the compounds: A simultaneous quantification method was designed and validated using the ICH Protocol. Using the best chromatographic conditions, the material was examined three times. Chromatograms were created for every sample, and the amount of each was noted along with the standard deviation and percentage RSD. The specificity, linearity, sensitivity, reliability, limit of detection, and limit of quantification of the RP-HPLC-developed method for detecting chemicals (TA and TAR) were found to meet standard criteria.

CONCLUSION

The results of this study demonstrated how geographic variation affects the phytochemical composition of extracts from *S. china*. The observed variations in TPC, TFC, and chemical composition suggest that while investigating the whole therapeutic potential of this medicinal plant, regional variation should be taken into account. To find the environmental and genetic elements that cause these regional variances, such as soil composition, climate, and altitude, as well as the conditions that encourage the production of desired plant chemicals, more research should be done. Chemical testing and GC-MS analysis qualitatively revealed the polar and nonpolar components present in the methanolic extract of *Smilax china*. The rhizome of *S. china* was used to isolate quercetin. In the concentration range of 0.1-0.8 µg/ml, chemicals TA and TAR showed excellent linearity. The present validated approach has an average recovery rate between 97% and 102%.

For the purpose of measuring the components TA and TAR in *S. china* extracts and other dietary supplements, a rapid reverse-phase HPLC method was created and verified. This method is timely, exact, and accurate.

CONFLICT OF INTEREST

This review article's authors have no conflicts of interest.

ACKNOWLEDGEMENT

We would like to sincerely thank the Professor .Dr. S. Dharmaraj Santhosam M. Pharm, PhD, St. John's College of Pharmaceutical Sciences and Research, Kattappana, Idukki district, Kerala, and Dr S. Ravichandran, M. Pharm, Ph.D., Professor and principal of St Mary's College of Medical Sciences, for granting us permission to conduct our review paper.

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HOW TO CITE: M. Maheshwari*, R. Srinivasan., A Critical Review on Phytochemical Analysis of *Smilax China* Rhizomes., J. Pharm. Sci., 2025, 1 (12), 414-423. <https://doi.org/10.5281/zenodo.17846237>

