

RESEARCH ARTICLE

Preliminary phytochemical screening of different solvent extracts of Root tuber of Smilax china

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ABSTRACT

Smilax china L. is indigenous to China and Japan but it is imported to India and is common in Indian bazaars. It is commonly known as Jin Gang Teng in Chinese, Chobchini in Hindi, and Madhusnuhi in Sanskrit and china root in English. It possesses anti-inflammatory, diuretic, anti-diabetic, anti-psoriatic, digestive properties. There is no authentic protocol for the Pharmacognostic parameters of Smilax china L. So, in the present study preliminary phytochemical screening of Smilax china was performed. Smilax china extracts were prepared using different solvent like chloroform, Acetone, N- hexane, ethanol, methanol and aqueous solvents using soxhlet apparatus. The phytochemical constituents present in these extracts were subjected to a variety of chemical tests. Methanolic extract showed higher affinity for various phytochemicals present in Smilax china

Key words: Smilax china, Phytochemical screening.

INTRODUCTION:

Smilax china L., popularly known as 'Jin Gang Teng' or 'Ba Qia', is widely used as a traditional Chinese medicine (TCM) for the treatment of diuretic and rheumatic arthritic conditions, as well as for detoxication, and to treat lumbago, gout, tumors, and inflammatory diseases; it is also used as a food in some areas of China [1]. The dried rhizome of *Smilax china L.* of the family Smilacaceae, known as Chobchini in Hindi, contains fat, sugar, glycoside, coloring matter, saponin, gum, tannin, cinchonin, smilacin and starch [2, 3] and it exhibits anti-inflammatory, diuretic, anti-diabetic, anti-psoriatic and digestive properties [4]. Steroidal saponins have been isolated from *Smilax riparia* and *Smilax china L.* and the anti-inflammatory activities of the isolated fractions have been investigated [5]. Free radical scavenging and antioxidant enzyme promoting activities were observed in the extracts of *Smilax china L.* root [6]. Supercritical fluid extraction has been used for the isolation of saponins from the tubers of *Smilax china L.* [7]. Anti-inflammatory and anti-nociceptive activities have been confirmed for the aqueous extract of *Smilax china L.* [8]. Steroidal saponins, isolated from *Smilax china L.* have been reported to possess anti-inflammatory activity [9]. Stilbenes and flavonoids in the extracts of *Smilax china L.* have been simultaneously determined using high performance liquid chromatography [10]. A flavonoid glycoside, isolated from the *Smilax china L.* rhizome,

exhibits in vitro anticancer effects on human cancer cell lines [11]. Kaempferol-7-O- β -D-glucoside was isolated from the *Smilax china L.* rhizome and this induced G2/M phase arrest and apoptosis in HeLa cells in a p53-independent manner [12]. High-speed counter-current chromatography has been used to isolate phenolic acids from *Smilax china L.* [13]. Anti-inflammatory activity of sieboldogenin, obtained from *Smilax china L.*, has been reported [14].

Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently en vogue in parts of the world [15]. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region [16]. Following such leads, plant parts are usually screened for phytochemicals that may be present. The presence of a phytochemical of interest may lead to its further isolation, purification and characterization. Then it can be used as the basis for a new pharmaceutical product. Various extracts of *Smilax china* possess various clinical effects. The present study was carried out to determine several pharmacognostic parameters and examine traditional claims for its various medicinal uses which can be proven scientifically.

MATERIALS AND METHODS:

2.1. PLANT MATERIAL:

The rhizomes of *Smilax china* L. were purchased from the Tirunelveli, Tamilnadu . The plant materials were authenticated by, National Institute of Siddha Medicine, Department of Botany , Tambaram , Chennai.

2.2. EXTRACTION AND PHYTOCHEMICAL INVESTIGATION:

The *Smilax china* L. rhizome was ground in a mixer and the powder, which passed through sieve no.10 and was retained on sieve no. 60, was used for preparation of the extract [15]. The drug was packed into a Soxhlet extractor and extracted. The phyto- constituents present in the extracts were subjected to a variety of chemical tests.

2.3 REAGENTS USED FOR DIFFERENT CHEMICAL TEST:

The following reagents were used for the different chemical group tests

a) Fehling's solution A (Copper sulphate solution)

34.64 gm copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml.

b) Fehling's solution B (Alkaline tartrate solution)

173 gm of sodium potassium tartrate and 50 gm of sodium hydroxide were dissolved in sufficient water to produce 500 ml. Equal volume of above solution were mixed at the time of use.

c) Mayer's reagent

1.358 gm of $HgCl_2$ was dissolved in 60 ml of water was mixed with a solution containing 5 gm of potassium iodide & the volume was adjusted by adding sufficient amount of distilled water to make it 100 ml.

d) Benedict's Reagent

With the aid of heat, 173 gm sodium citrate and 100 gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 800 ml with water. After filtration the solution was diluted to 850 ml. 17.3 gm of $CuSO_4 \cdot 5H_2O$ was dissolved in 100 ml distilled water. Then the two solutions was mixed with constant stirring & made upto 1000 ml.

e) Molish Reagent (α -naphtha solution)

15 gm of pure α -naphtha was dissolved in 100 ml of ethanol or chloroform.

f) Salkowski reagent: Chloroform & a few drops of concentrated sulphuric acid.

g) Libermann-burchard reagent: Chloroform & few drops of concentrated sulphuric acid & 2-3 drops of acetic anhydride.

h) Ferric chloride (5%): 5 gm ferric chloride in 100 ml distilled water.

i) Potassium dichromate (10%): 10 gm Potassium dichromate in 100 ml distilled water.

j) Dragendroff's reagent: 8 gm bismuth nitrate is dissolved in 20 ml of concentrate nitric acid & 27.2 gm of potassium iodide in 50 ml of distilled water. The two

solutions are mixed & allowed to stand. When potassium nitrate crystallizes out, the supernatant is decanted off and make up to 100 ml with distilled water.

k) Wagner's reagent: (Iodo-potassium iodide solution) 2gm of iodine & 6 gm of potassium iodide in 100 ml of water.

l) Hager's reagent: (picric acid solution) 1 gm of picric acid in 100 ml of water.

TESTS PROCEDURE FOR IDENTIFYING DIFFERENT CHEMICAL GROUPS 17-19

The following tests were performed for identifying different chemical groups

Tests for reducing sugar

(a) Benedict's test

0.5 ml of aqueous extract of the plant material was taken in a test tube. 5 ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously.

(b) Fehling's Test (Standard Test)

2 ml of an aqueous extract of the plant material was added to 1 ml of a mixture of equal volumes of Fehling's solutions A and B and was boiled for few minutes.

Test for carbohydrates & gums

Molish test:

5 ml sample was taken and then a few drops of Molish reagent were added. Then the tube was inclined and 1 ml of sulphuric acid was added gradually at the bottom of the test tube through one side.

Test for alkaloids

(a) Mayer's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent was added.

(b) Dragendroff's test

2 ml sample and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendroff's reagent was added.

(c) Wagner's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of iodine solution was added.

(d) Hager's test

2 ml sample and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of picric acid was added.

Tests for tannins

(a) Ferric Chloride Test

5 ml solution of the extract was taken in a test tube. Then 1 ml of 5% Ferric chloride solution was added.

b) Potassium dichromate test

5 ml solution of the extract was taken in a test tube. Then 1 ml of 10% Potassium dichromate solution was added.

Test for steroids

(a) Salkowski reaction

A few mg of sample was dissolved in chloroform and a few drops of concentrated sulphuric acid are added to the solution.

(b) Libermann-burchard reaction

A few mg of sample was dissolved in chloroform and a few drops of concentrated sulphuric acid are added to the solution followed by the addition of 2-3 drops of acetic anhydride.

Test for flavonoids

A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material.

Test for Saponins

1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes.

RESULTS AND DISCUSSION:

Extract yield of Smilax china L.

Extract yield for the rhizome of Smilax china L. was determined in these solvents are seems to higher in Methanolic extract, followed by ethanolic , Aqueous , Chloroform , N-hexane and Acetone extracts respectively. TLC and HPLC analysis with chemical controls may be conducted in further studies to provide detailed phytochemical information.

Percentage yield value of extract from Smilax china

Table 1: Showing percentage yield value of extracts of Smilax china

Solvent	Powder taken for extraction	Weight of the beaker	Weight of beaker with extract	Weight of the extract obtained	% of yield of methanol extract
Acetone	125gm	42.70gm	51.24gm	8.54gm	6.832gm
Ethanol			53.04gm	10.34gm	8.272gm
Methanol			53.48gm	10.78gm	8.624gm
Aqueous			51.78gm	9.08gm	7.264gm
Chloroform			51.67gm	8.97gm	7.176gm
N-Hexane			50.76gm	8.06gm	6.448gm

% of yield of methanol extract = (Weight of extract/Powder taken for extraction) x 100

Table.No.2 Preliminary Phytochemical Tests of Smilax china

GROUP TEST	NAME OF THE TEST	OBSERVATION					
		Acetone	Ethanollic extract	Methanolic extract	Aqueous	CHCL3	N-hexane
Carbohydrate & Gums	Molish Test	+	+	+	+	-	+
Reducing Sugar	Fehling's Solution Test	+	+	+	+	-	-
	Benedict's Test	+	+	+	+	-	-
Alkaloids	Mayer's Test	+	+	+	-	+	+
	Dragendroff's Test	+	-	-	+	+	+
	Wagner's Test	+	+	+	-	+	+
	Hagner's Test	+	-	+	+	+	+
Steroides	Salkowski reaction	+	+	+	+	+	+
	Liebermann-burchared reaction	+	+	+	+	+	+
Glycosides	Salkowski reaction	-	-	+	-	-	+
	Liebermann-burchared reaction	-	-	+	-	-	+
Tannins	Ferric chloride test	-	-	+	-	+	-
	Potassium dichromate test	-	-	+	-	+	-
	Keller-Kiliani Test	-	-	+	-	+	+
Flavonoids	Hydrochloric acid Test	+	-	+	-	-	-
Saponins	Foam Test	-	+	+	-	-	-

+ = present, - = absent

Results obtained for qualitative screening of phytochemicals in root tuber of smilax china are present in table 2. of the nine phytochemicals screened for, four were found present in various solvent extracts. They are steroid, carbohydrates, reducing sugar and alkaloid. Remarkably, flavonoids and saponins were not present in root tuber of smilax china. According to Tiwari et al., the factors affecting the choice of solvent are; quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of

inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractant (20). The logic in using different solvents when screening for phytochemicals in plant materials was clearly validated in this study. The result indicates that *Smilax china* roots promises as source of pharmaceutically important phytochemicals. Alkaloids generally present in root tuber play some metabolic role and control development in living system. They are also

involved in protective function in animals and are used as medicine especially the steroidal alkaloids. Tannins are known. The flavonoids and phenolic compounds in plant have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc.

CONCLUSION:

Phytochemicals found present in root extracts of *Smilax china* indicates their potential as a source of principles that may supply novel medicines. Further studies are therefore suggested to ascertain their antimicrobial, antiplasmodic and fertility activities. Furthermore, isolation purification and characterization of the phytochemicals found present will make interesting studies.

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