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Research Article

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Pharmacognostical and phytochemical studies on leaves of Stephania japonica Linn.

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ABSTRACT

The aim of the present study was to assess the Pharmacognostic and Phytochemical studies on Leaves of Stephania japonica Linn. The objective of the present work comprise of collection, identification, microscopical and phytochemical evaluation of Leaves of Stephania japonica Linn. The leaves section were taken and cellular structures were studied. The T.S of Leaves shows the presence of epidermis, vascular bundle, veinislet, veintermination, spongy mesophyll, xylem, phloem,sclerenchyma,glandular and non glandular trichomes. The phytochemical studies indicate the Ash value, Extractive value, Crude fibre content, Fluorescence characters and the Preliminary phytochemical tests on various extracts indicates the presence of alkaloids, glycosides, flavanoids, saponins, carbohydrates, tannins, phenols, and mucilage.

Keywords: Stephania japonica, Menispermaceae, alkaloid,

INTRODUCTION

Stephania japonica Linn. (Family- Menispermiaceae) is a slender wiry climber. Leaves peltate thinly papyraceous, glabrous on both the surfaces, broadly triangular, ovate- acuminate, 3-12 cm long, apex acutely acuminate or obtuse, base rounded, margins entire. Inflorescence axillary, compound umbelifer cymes, usually single per axil, 3-6 cm long. Found throughout India upto an altitude of 2100m. The species is common as an undergrowth in the Asia to southern Pacific elsewhere.

EXPERIMENTAL SECTION

Microscopical study of the Leaves Plant Collection & Authentication

The fresh plant material was collected from Trichy in the month of June 2010. The plant material was taxonomically authentified by Dr. Jayaraman, Botanist, Chennai, Tamil Nadu. Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin-5ml + Acetic acid- 5 ml +70% ethyl alcohol-90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary – butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point $58 - 60^{\circ}$ C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks (Johansen, 1940; O'Brien, *et al.* 1964).

Sectioning

The paraffin embedded specimens were sectioned with the help of rotary Microtome. The thickness of the sections was $10 - 12 \mu m$. Dewaxing of the sections was done by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue (O'Brien, *et al.* 1964). Since toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc., wherever necessary sections were also stained with safranin and Fast – green and IKI for starch.

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared (Sass, 1940). Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerin medium after staining. Different cell components were studied and measured (Metcalfe, C.R. 1950).

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale- bars. Descriptive terms of the anatomical features are as given in the standard anatomy books (Esau, 1964).

Identification of Plant Constituents by Phytochemical Tests (Kokate, C.K. et al. 1994)

The powdered drug and various extracts of the plant *Stephania japonica* was subjected to phytochemical tests for identification of its active constituents.

Test for Alkaloids

A small portion of solvent free extracts were stirred separately with few drops of dilute hydrochloric acid and filtered & tested carefully with various alkaloidal reagents.

Mayer's reagent	-	Cream precipitate
Dragendroff's reagent	-	Orange brown precipitate
Hager's reagent	-	Yellow precipitate
Wagner's reagent	-	Reddish brown precipitate

Test for Carbohydrates & Glycosides

The minimum amount of extracts were dissolved in 5ml of distilled water & filtered. The filtrate was subjected to test for carbohydrates & glycosides.

a)Molisch's test

The filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol & 2ml concentrated sulphuric acid was added along the sides of test tube. Violet ring was observed at the junction of 2 layers which showed the presence of carbohydrate.

Test for Glycosides:

a)Legal's test

To the hydrolysate 1 ml of sodium nitroprusside solution was added & then it was made alkaline with sodium nitroprusside. Pink colour was produced and it indicated the presence of glycosides.

b)Borntrager's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. No colour change in ammonical layer was observed.

Test for Phytosterol:

1 g of different extracts were dissolved in few drops of dilute acetic acid; 3ml of acetic anhydride was added followed by few drops of concentrated sulphuric acid. Appearance of bluish green colour showed the presence of phytosterol.

Test for Fixed Oil & Fats

a) Small quantities of various extracts were separately pressed between two filter papers. No oil stain on the paper indicated the absence of fixed oil.

b)Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. Mixture was heated on water bath for 1-2 hours. No soap formation, neutralization of alkali indicated the absence of fixed oil and fats.

Test for Lignin:

With alcoholic solution of phloroglucinol and hydrochloric acid the appearance of red colour showed the presence of lignin.

Test for Saponins:

The extracts were diluted with 20ml of distilled water and it was agitated on graduated cylinder for 15 minutes. The presence of saponins was indicated by formation of 1cm layer of foam.

Test for Tannins and Phenolic Compounds:

Small quantities of various extracts were taken separately in water and tested for presence of phenolic compounds and treated with

1)Dilute ferric chloride solution (5%) - violet colour

2)1% sodium gelatin containing 10% sodium chloride - white precipitate

3)10% lead acetate solution – white precipitate

Test for Proteins and Free Amino Acids:

Dissolved small quantities of various extracts in few ml of water and treated with.

1. Millon's reagent – Red colour showed the presence of proteins and free amino acids

2. Ninhydrin reagent – Purple colour showed the presence of proteins and free amino acids.

3. **Biuret test** – Equal volume of 5% solution and 1% copper sulphate solutions were added. Appearance of purple colour showed the presence of proteins and free amino acids.

Test for Gums and Mucilage:

Powdered drug was treated with ruthenium red solution. No characteristic colour change was obtained indicating absence of gums & mucilage.

Test for Flavonoids :

a) With aqueous sodium hydroxide solution - blue to violet colour (Anthocyanins); yellow colour (Flavones); yellow to orange colour (Flavones)

b)With concentrated sulphuric acid – yellowish orange colour (Anthocyanins); yellow to orange colour (Flavones); orange to crimson (Flavones)

c)Shinoda's test : Test extracts were dissolved in alcohol, to that piece of magnesium turnings followed by concentrated hydrochloric acid were added drop wise and heated. Appearance of magenta colour showed the presence of flavonoids.

RESULTS AND DISCUSSION

Macro and microscopic characters

The plant Stephania japonica is a slender wiry climber, Leaves peltate thinly papyraceous, glabrous on both the surfaces, broadly triangular, ovate- acuminate 3-12 cm long, apex acutely acuminate or obtuse, base rounded , margin entire. Flowers small, male flower greenish white (or) Yellowish. Drupes light yellow to orange red, obovate, glabrous.











Fig - 2











Fig. 1-Stephania japonica Leaves, Fig.2-Flowers, Fig.3-Seed, Fig.4-fruits, Fig.5-Leaves dorsal view, Fig.6-Leaves ventral view

Microscopic features

Lamina(Fig.7)The lamina in distinctly dorsiventral measuring about 500 µm thick. The adaxial epidermis, tabular in shape and horizountally extended narrow cells with even surface. The abaxial epidermal layer consist of dilated hemispherical thin walled papillate cells. Which are 15µm thick.. The mesophyll tissue is differentiated in to adaxial zone of palisade cells and abaxial spongy parenchyma. The palisade tissue consists of two layers of cylindrical

cells, the upper row of cells are compact and the lower of cells are loosely arranged. (Fig.7) The spongyparanchyma cells are five or six layered, the cells are lobed and inter connected with each other forming wide air chambers. The vascular strands of the lateral veins are situated in the median part of the lamina, ensheathed by bundle sheaths parenchyma.

Midrib (Fig.7) In cross sectional view, the midrib appear abaxially semicirhump. It is 1.5mm thick and 1.2mm wide. The epidermis of the midrib consists small elliptical thick walled cells. The ground tissue includes two or three layers of thick walled angular cells towards the periphery and remaining portion includes compact thin walled parenchyma cells.

The vascular strand of the midrib is single, collateral and thick.. It is placed in the central part of the midrib. A mass of wide, circular thick xylem elements which are up to $30\mu m$ wide. Phloem occurs in thick horizontal block beneath in xylem. These are two, thick ares of sclerenchyma cells, one on the upper and the other on the lower ends of the vascular strand. The sclerenchyma cells are wide, angular and have thick lignified walls.

Venation of the lamina(Fig.8)

Lamina was cleared to study the venation pattern from the surface view. The primary and secondary veins form dense reticulate venation. The viens are thin and straight. The veinislets are wide and have distdrict veins boundaries. The islets are variable in shape and are random in orientation.

Vein terminations are present in most of the vein islets. The terminations are unbranched or branched once or twice. The terminations are thin and glandular.

Petiole(Fig.8) : The petiole is circular in transactional outline. It is 1mm thick. These is a wide circular Central canal formed by the lysis of the pith parenchyma. The epidermal layer is thin , the cells being spindle shaped with thicker outer tangitial walls(Fig.8) inner to the epidermis is a wide paranely in atous ground tissue compresing 6 or 7 layers of angular, thick walled copact cells.

The vascular strands have five to ten, wide, angular solitary thick walled xylem elements and wide mass of Phloem elements. The Vascular – curcle is enclosed with in a continuous wavy cylinder is 4- cells in thickness; the cells are thick walled and lignified with narrow cell lemeu.

Powder Microscopic Observation

The powder of the plant includes the following inclusious.

1. Small fragments of leaf are often seen in the powder. When seen in face view, the leaf fragment exhibit e veinterminations which are associated with long, wide lobed sclereids. These foliar- sclereids have thick walls, wide lumen and numerous simple pits.

2. Epidermal trichomes (Fig.9)

Unicellular, unbranched, non glandular trichomes are common in the powder they are heavily thick walled with wide lumem. The surface of the trichome is smooth. It is thin uniform in thickness and gradually tapers at the tip. The trichome is 350μ m long and 25μ m thick at the base.



Fig -7 T.S of Midrib







Fig -11 A fibre



Fig -12 Cross section of the petiole entire view



VB CC Ph Se Se

Table - 1 Data showing the Physico - Chemical standards of leaves powder of Stephania Japonica

S.No	Parameters	Average
1.	Total Ash %w/w	7.79
2.	Acid In Soluble Ash%w/w	2.23
3.	Water soluble Ash%w/w	9.12
4.	Sulphated Ash%w/w	8.75
4.	Loss on Drying%w/w	8.75
5.	Water Soluble Extractive%w/w	1.52
6.	Alcohol Soluble Extractive%w/w	9.19
7.	Crude Fibre Content%w/w	9.93

3. Also seen in the powder are adaxial epidermal peeling and abaxial abaxial epidermal peelings:

The adaxial epidermal peeling is apostomatic (with out stomata). The cells are fairly wide and amoeboid in outline due to wavy auticlinal walls. The abaxial epidermal peeling is stomatiferous. The epidermal cells are wide with less wavy anticlinal walls. The stomata are small and narmoly elliptical.

4. Thick pieces of lamina are seen in surface view showing venation and trichomes (Fig.9). The venation is reticulate. The vein islets are wide and district. They are with or with out vein terminations.

5. **Glandular trichomes** (Fig.10) Thick, spherical, short stalked glandular trichomes are sparsely seen in the powder. It consists of multicellular body with darkly stained cells. The shalk is very short and in unicellular body part of the trichome is 150µm thick.

6. **Fiberes.** Xylem fiberes are frequent in the powder. They are long, narrow with tapering ends. The walls are thick and the lumen is fairy wide.

Baaganta	Petroleum ether extract		Chloroform extract		Acetone extract		Ethanol extract		Aqueous extract		Drug powder	
Reagents	DL	UVL	DL	UVL	DL	UVL	DL	UVL	DL	UVL	DL	UVL
Extract as such	Green	Green	Yellow	Yellow	Greenish yellow	Light green	Brownish yellow	Brownish yellow	Dark brown	Dark green	-	-
1 N Sodium Hydroxide (aqueous)	Yellowish green	Dark green	Yellowish brown	Dark green	Yellowish brown	Green	Yellow	Yellow	Yellowish brown	Greenish yellow	Pale green	Yellow wish green
1 N Sodium Hydroxide (alchohol)	Yellowish green	Yellowish brown	Yellow	Yellow colour	Brown	Greenish black	Yellowish brown	Pale green	Brownish yellow	Greenish brown	Green	Fluorescent green
1 N Hydrochloric Acid	Pale green	Green	Brown	Green colour	Yellowish black	Brownish green	Yellowish brown	Brownish yellow	Pale yellow	Pale yellow	Brown	Light green
50% Nitric Acid	Dark green	Brownish yellow	Greenish yellow	Yellow colour	Yellowish black	Yellowish black	Brownish black	Brownish green	Pale yellow	Greenish yellow	Green	Flu Florescent green
50% Sulphuric Acid	Pale yellow	Yellow	Green	Yellowish green	Brownish green	Pale yellow	Pale brown	Yellowish green	Whitish yellow	Pale green	Green	Fluorescent green
Methanol	Brownish green	Brown	Light green	Green	Greenish yellow	Yellowish green	Dark brown	Brownish green	Pale yellow	Yellow	Green	Fluorescent green
Ammonia	Pale brown	Greenish brown	Greenish yellow	Dark green	Green	Green	Greenish yellow	Dark green	Brownish yellow	Dark green	Green	Yellowish green
Iodine	Reddish brown	Yellowish brown	Brownish yellow	Yellowish brown	Reddish yellow	Greenish yellow	Yellowish brown	Pale green	Black	Brownish green	Brown	Light green
Ferric Chloride	Dark brown	Dark green	Greenish brown	Yellow	Black colour	Black	Black	Black	Dark brown	Blackish green	Green	Light green

Table – 2 Florescence Analysis of Extracts and Drug powder of Stephania japonica

Table- 3 Successive Solvent Extraction of Stephania japonica

S. No	Extracts	Colour and Consistency	Percentage yield of extracts
1.	Petroleum ether $(60 - 80\%)$	Yellowish colour	1.57
2.	Chloroform	Dark Green	0.76
3.	Acetone	Darkgreen colour.	1.21
4.	Ethanol	Green colour	1.99
5.	Aqueous	Brownish green colour	2.41

Quantitative standards of powdered Leaves of Stephania japonica:

Ash values including total ash, acid insoluble ash and water soluble ash of fruit powder were done as per Indian Pharmacopoeia. The results are shown in Table 1.Successive extractive value with different solvents with leaves powder were done. The colour, consistency and percentage of extractive values shown in Table 3. The fluorescence analysis of powdered drug as well as its extractives in daylight and UV light were examined. The observations are given in Table 4. The presence or absence of different pytoconstituents, viz. alkaloids, carbohydrate, glycosides, flavanoids, terpenoids, saponins and phenolic compounds were detected by prescribed methosds and results are given in Table 5.

Table- 4 Data showing the preliminary phytochemical screening of Stephania Japonica

Phytoconstituents	Petroleum ether extract	Chloroform extract	Acetone extract	Ethanol extract	Aqueous extract
Alkaloids	(-)	(+)	(+)	(+)	(+)
Carbohydrates	(-)	(+)	(+)	(+)	(-)
Glycosides	(-)	(+)	(+)	(+)	(-)
Flavonoids	(-)	(+)	(+)	(+)	(-)
Phytosterols	(+)	(+)	(+)	(+)	(+)
Fixed oils and Fats	(-)	(+)	(-)	(-)	(-)
Saponins	(-)	(-)	(-)	(-)	(-)
Phenolic compounds and Tannins	(-)	(+)	(+)	(+)	(+)
Lignins	(+)	(+)	(+)	(+)	(+)
Proteins and free Amino acids	(+)	(+)	(+)	(+)	(+)
Gums and Mucilage	(-)	(-)	(+)	(-)	(+)
(+) Positive				(-) Negative	

(+) Positive

(-) Negative

CONCLUSION

The major problem faced in herbal formulation industry is the identification of authenticated raw material and in the absence of data one can use adulterant in the drug formulation. The results abtained in morphological studies reported here in established the macro & microscopic parameters that characterize the plant Stephania japonica Linn. Family (Menispermaceae). Those macroscopical characteristics can be utilized for quick identification of the drug and are particularly useful in the case of powder materials.

Present study concludes that physiochemical parameters, Qualitative and Quantitative analysis may be used for Quality control parameters of *Stephania japonica* to obtain genunine and standard drug for therapeutic purpose.

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