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A Study on Pharmacognosticaland Phytochemical Evaluation of Leaves of *Passiflora nepalensis* wall

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Abstract: Now-a-days, herbal medication is proving to be better and safer mode of therapy of various diseases, without exhibiting any adverse effects as those with the allopathic medication. *Passifloranepalensis*belongs to the family (Passifloraceae family). It is commonly known as Passion flower. The plant is a glabrous climber with slender branches and distant leaves growing in shady areas. *Passifloranepalensis*is distributed throughout Sikkim, Himalayan region of India, at about 4000-6000 ft. The present study provides updated information on its pharmacognostic, phytochemical analysis and pharmacological properties. Transverse sections of *Passifloranepalensisleaf* showeddorsiventral leaf structure. Midrib region showed layers of thick walled collenchyma cells below the upper epidermis & above the lower epidermis along with highly lignified vascular bundles. The flower and tender shoots has sedative, antispasmodic and tranquilizing activity. Physicochemical studies shows, total moisture content (8.33%), total ash value (13.33%), acid insoluble ash value (16.66%), alcohol soluble extractive values (96%) and water soluble extractive values (40%). Preliminary phytochemical analysis (organic analysis) revealed alkaloids, carbohydrates, glycosides, saponins, proteins, phenolic compounds and flavonoids are present. The other variety of this species having several important constituents like apigenin and isovitexin flavonoids, cyanogenic glycosides and indole alkaloids (harman). As there is no detailed work reported in leaf, therefore pharmacognostical evaluation including physicochemical parameters, preliminary phytochemical standards were determined .The study revealed specific identities for the plant, which will be useful in identification, as a control to abet adulterants and for future standardization work.

Key words: *Passiflora nepalensis* Wall · Phytochemical Studies · Pharmcognostical Evaluation

INTRODUCTION

The genus Passiflora consists of 500 species which are mostly found in warm and tropical regions. Passiflora comes from Latin word "Passio" that was first time discovered by Spanish discoverers in 1529 and was described as a symbol for "Passion of Christ" [1, 2] . This plant was used widely in traditional medicine in West India, Mexico, Netherland, South America, Italia and Argentina. One of species of this genus named as *Passifloranepalensis*Walp. (Passifloraceae) is more popular than its other species in Eastern India. *Passifloranepalensis* is used in folklore medicine for treating hypertension and inflammation [3]. *Passifloranepalensis* (Passion flower Family) is a wide spreading climber, grown frequently in gardens as an ornamental. Stems wiry, leaves three lobed and serrate, flower pale pink in color and fruits ovoid or globose. This is a fine climber suitable for covering arbours, verandahs and arches. It can be propagated by seed or layering. The fruit is edible when ripe [4]. Its medicinal usage has been reported in the traditional systems of medicine such as Ayurveda, Siddha and Unani. Passiflora contains several compounds including alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds [2, 3, 5]. Antihypertensive and negative chronotropic effects of

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Passifloranepalensis have been investigated earlier. Furthermore, it has been traditionally claimed by rural community of Sikkim State for its lipid lowering property and therapeutic importance in cardiovascular disorders. Its folkloric use and antihypertensive effect due to protection against renal ischemia/reperfusion have also been established [6].

MATERIALS AND METHODS

Plant Material: The whole plant of *Passifloranepalensis* were collected in the month of October from the Eastern part of India (Sikkim Himalayas). The Herbarium specimen (No. 168) of plant was deposited in the Department of Pharmacognosy and it has been identified from Himalayan Pharmacy Institute, Majhitar. The macroscopical observations were carried out [7]. The microscopical investigations, histochemical tests, stomatal index, vein islet number were performed [7, 8].

For microscopical studies free hand sections were taken and studies were undertaken as per standard guidelines. Phluroglucinol and conc. HCl (1:1), iodine solution was used for differential staining [7].

The whole plant was dried in shade and powdered (No. 60 mesh) and 3.5274 oz of the dried powder was Soxhletextracted successively with petroleum ether, benzene, chloroform, acetone, ethyl acetate and methanol. Physicochemical standardization methods including determination of moisture content (loss on drying), determination of total ash and acid insoluble ash, extractive values were carried out as per WHO recommendations and authentic procedures mention in Ayurvedic pharmacopoeia of India. Fluorescence studies were carried out by treating 0.5 gm of powdered drug with different reagents and observation in color was made in visible light, U.Vlight of short (254nm) and long wavelength (365nm) under U.V chamber. Photomicrographs were obtained by compound binocular microscope OLYMPUS BX41and photomicrography was done using Olympus C7070 camera.

RESULTS AND DISCUSSION

Macroscopical Characters: The macroscopical studies showed that the plant is the glabrous climber with slenderbranches &distant leaves. It also revealed the shape of leaves as palmately 3-lobed, the lobes ovate-lanceolate with entire margin, slight bitter in taste & size varying from 5-12 cm long.

Fig. 1: A climberof *Passifloranepalensis*

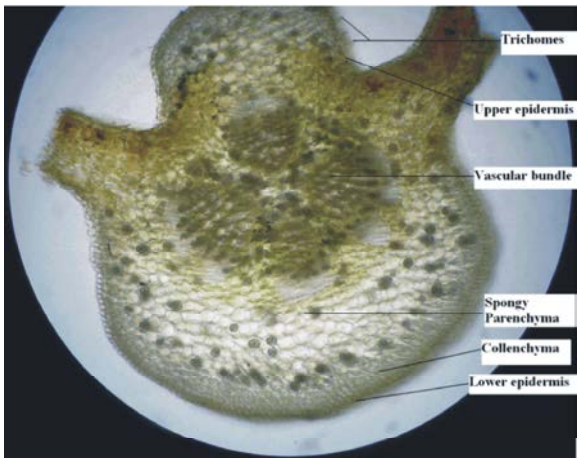

Fig. 2: T.S. of Leaf

Powder Characteristics: The powder microscopy revealed the presence of unicellular, non lignified covering trichomes, prismatic type calcium oxalate crystals, ranunculaceous stomata, simple starch grains, &scalariform vessels.

Microscopy Characteristics: Mesophyll showed dorsiventral leaf structure. Midrib region showed layers of thick walled collenchyma cells below the upper epidermis & above the lower epidermis along with highly lignified vascular bundles.

Quantitative Microscopy: It revealed the presence of stomata in both upper & lower epidermis.

Pharmacognostic Evaluation of the Plant: Air dried material was used for quantitative determination of physicochemical values. Ash value, Loss on drying, extractive values, foaming index, foreign organic matter were also performed.

Determination of Ash Value

Total Ash Value: Two grams of dried and powdered plant material was taken in the pre-weighed clean sintered silica crucibles. Then, they were incinerated by gradually increasing of the temperature (400-500°C) in the muffle furnace till white ash obtained until constant weight of ash obtained. The crucible was cooled to room temperature in a desecrator and weighed the ash and calculated the % of total ash with reference to air dried sample of the crude drug using following formula:

$$\text{Total Value (\%)} = \frac{\text{Z- X}}{\text{Y}}\ 100$$

where, Z= Weight of the crucible; X = Weight of the crucible with ash; Y = Weight of the powder taken (g) [9].

Acid Insoluble Ash Value: The total ash content of the plant material obtained was boiled for 15 min, after adding 25ml of 25 % (v/v) HCl in to a 100 ml beaker and was allowed to cool. It was then filtered through a Whatman filter paper No. 44 (ash less) and wash the residue twice with hot water. The insoluble ash thus retained on filter paper along with paper was ignited in a preweighed sintered crucible (1000°C). Then the crucible along with the residue was weighed and calculated the acid insoluble ash content using the following formula:

$$\textit{Acid insoluble ash Value}\ (\%) = \frac{a}{Y} \times 100$$

where
a = Weight of the residue;
Y= Weight of powder taken (g) [10]

Water Soluble Ash Value: The total ash value was determined using 2 g of the air-dried powdered sample. The total ash was boiled for 5 minutes with 25 ml of distilled water; the insoluble matter was collected on an ash less filter paper, washed with hot distilled water and ignited for 15 minutes at temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the total ash; the difference in weight represents the water-soluble ash. The percentage of the water-soluble ash was calculated with reference to the air-dried powdered plant sample. It was calculated by using following formula:

$$\textit{Water insoluble ash Value}\ (\%) = \frac{a}{Y} \times 100$$

where

a = Weight of the residue;
Y= Weight of powder taken (g)

Water soluble ash Values (%) = Total ash value - Water insoluble ash value [9]

Determination of Extractive Values and Organoleptic Studies of Crude Extract: For the determination of Organoleptic characters such as color, nature, taste and yield of the extracts, 100g of dried and powdered plantmaterial was successively extracted in the soxhlet extractor using petroleum ether, chloroform, ethanol (99%, v/v) and distilled water solvents in the increasing order of polarity for 24h. The Resulting liquid extracts were evaporated to dryness under reduced pressure. The yield of the extracts was calculated using the following formula:

$$\textit{Weight of the Extractive value}\ \% = \frac{\text{Residue} \times 100}{\textit{Weight of the plant material taken}}$$ [10]

Water Soluble Extractive Value: 5 gm coarse and air dried drug material was macerated with 100 ml water in a stoppered flask for 24 hrs with frequent shaking for first 6 hrs. The extract was filtered rapidly through filter paper taking precaution to prevent excessive loss of solvent.The residue was evaporated in a flat bottom shallow dish, dried at 105°C weighed and kept in a desiccator. Average extractive value in Percentage w/w (on dry weight basis) was calculated with reference to air dried drug [11].

Chloroform Soluble Extractive Value: Accurately weighed 5 gmcoarse and air dried powdered drug was macerated with 100ml chloroform in a stoppered flask for 24 hrs with frequent shaking for 6 hrs. It was then filtered rapidly through filter paper taking precautions to prevent excessive loss of chloroform. The volume was made up to100ml with chloroform. The residue was evaporated in a flat bottom shallow dish, dried at 105°C, weighed and kept in desiccators. Average extractive value in percentage w/w (on dry basis) was calculated with reference to air dried drug.

Petroleum Ether Soluble Extractive Value: A 5 gmcoarse and air dried drug material was macerated with 100ml petroleum ether in a stoppered flask for 24 hrs with frequent shaking for first 6 hrs. The extract was then

Table 1.Ash values of *Passifloranepalensis Wall.*

Sl. No.		% w/w
1	Total ash	13.33
2	Acid insoluble ash	16.66
3	Water insoluble ash	6.66

Table 2; Extractive values of *Passifloranepalensis Wall*

Sl. No.	Extracts	Extractive value (% w/w)
1	Water soluble extracts	40
2	Alcohol soluble extracts	96

Table 3: Moisture Content of *Passifloranepalensis Wall*

Fresh weight (gm)	Dry weight (gm)	Loss on drying (gm)	Moisture content (%)
3	2.75	0.25	8.33

Table 4: Stomatal Index of *Passifloranepalensis Wall*

		Stomatal index
Sl. No.	Species	Upper surface
1	*P. nepalensis*	31.57

Table 5: Vein-islet & Vein termination Numbers of *Passifloranepalensis Wall*

Sl. No.	Species	Range of vein-islet number	Range of vein termination number
1	*P. nepalensis*	14-16	3-7

filtered rapidly through filter paper taking precaution to prevent excessive loss of solvent. The residue was evaporated in a flat bottom shallow dish, dried at 105°C weighed and kept in a desiccators. Average extractive value in Percentage w/w (on dry weight basis) was calculated with reference to air dried drug [10].

Fluorescent Studies of Powder Drugs: A lot of herbs show fluorescence when the cut surface or powder is exposed to UV light and this can be useful in their identification. The fluorescence character of the plant powders (40 mesh) was studied both in daylight and UVlight (254 nm and 366 nm) and after treatment with different reagents like sodium hydroxide, hydrochloric acid, nitric acid and ferric chloride etc. [12,13].

Phytochemical Screening: The entire plant was collected and dried in shade and reduced to coarse powder. The powdered material was extracted with petroleum ether, chloroform, ethanol and water in Soxhlet apparatus. The extract was filtered hot and solvent removed by distillation under reduced pressure. The percentage yield was calculated and the extract was further subjected to phytochemical tests for Alkaloids, Glycosides, Flavonoids and Tannins.

Table 6: Fluorescence studies of *Passifloranepalensis Wall*

		Ultra -Violet Light	
Treatment of powder	Visible rays	Short Wave (254 nm)	Long wave (365 nm)
Powder as such	Dirty green	Dark green	Black
Powder + 50% H_2SO_4	Dark green	Dark green	Black
Powder + 50% HNO_3	Brown	Dark green	Black
Powder + 5% KOH	Bright green	Dark green	Black
Powder + Methanol	Yellowish green	Dark green	Black
Powder + 1N HCl	Brown	Dark green	Black
Powder + 1N MethanolicNaOH	Yellowish green	Dark green	Black

Table 7: Successive Solvent Extractive Values & Nature of Extracts

Sl. No.	Solvent	Color	Consistency	Extractive value (%w/w)
1.	Petroleum ether	Black	Sticky	1.008
2.	Benzene	Black	Sticky	1.006
3.	Chloroform	Black	Sticky	0.775
4.	Acetone	Dark brown	Sticky	1.25
5.	Ethyl acetate	Dark brown	Sticky	1
6.	Methanol	Dark brown	Semi-solid	6.666
7.	Ethanol	Dark brown	Semi-solid	2.725
8.	Chloroform water	Blackish brown	Solid	9.908

Table 8: Phytochemical evaluation of *Passifloranepalensis* extract

Sl. No.	Phytochemical test	Pet. Ether extract	Benzene extract	Chloroform extract	Acetone extract	Ethyl acetate extract	Methanol extract	Ethanol extract	Aqueous extract
1	Alkaloids								
	a. Mayer's test	-	-	-	+	-	+	+	+
	b. Wagner's test	-	-	-	+	-	+	+	+
	c. Hager's test	-	-	-	+	-	+	+	+
	d. Dragendorff's test	-	-	-	+	-	+	+	+
2	Carbohydrates &Glycosides								
	a. Molish's test	-	-	-	-	+	+	+	+
	b. Fehling's test	-	-	-	-	-	-	-	-
	c. Barfoed's test	-	-	-	-	-	-	-	-
	d. Benedict's test	-	-	-	-	-	-	-	-
	e. Legal's test	-	-	-	+	-	+	-	+
	f. Kellar-Killiani test	-	-	-	+	+	+	+	+
3	Saponins Foam test	-	+	+	+	-	+	+	+
4	Proteins &Amino acids								
	a. Millon's test	-	-	-	-	-	-	-	-
	b. Biuret test	-	-	-	-	-	-	-	+
	c. Ninhydrin test	-	-	-	-	-	-	-	-
5	Phytosteroids								
	Liebermann-Burchard's test	-	-	-	-	-	-	-	-
6	Fixed oils &fats								
	a. Spot test	-	-	-	-	-	-	-	-
	b. Saponification test	+	+	+	+	-	-	+	-
7	Phenolic compounds &flavonoids								
	a. Ferric chloride test	-	-	-	+	-	+	+	-
	b. Gelatin test	-	-	-	-	-	-	-	-
	c. Lead acetate test	-	-	-	+	-	+	-	+
	d. Alkaline reagent	-	-	-	+	-	+	-	-
	e. Mg and HCl red.	-	-	-	+	+	+	-	+
8	Gums &Mucilages								
	Alcohol 95% test	+	-	-	-	-	-	-	-

CONCLUSIONS

Passifloranepalensis belonging to the family Passifloraceae is one of the important medicinal plants. An attempt has been made to evaluate the pharmacognostical, phytochemical parameters. The identification of the plant material taxonomically and pharmacognostically is important to provide pharmacognostical standards and also to avoid spurious or adulterated drugs. In the present study, the taxonomical characters dealing with the exomorphology of the plant have been studied which help in identification of the plant in the field. The microscopical and phytochemical studies help in evolving diagnostic characters for the identification of the drug. The different parts of the plant can be identified by the macro- and microscopical characters. The physicochemical constants like moisture content, ash values, extractive values, stomatal index, vein-islet number were determined. These help in formulating pharmacopoeial standards for the drug.

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