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Research Article

RP-HPLC for the isolation of *C*-glycosylflavones from methanolic extract of *Passiflora nepalensis* Wall., Passifloraceae

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Abstract

Plants from the genus *Passiflora* have been used in traditional medicine by many cultures. Flavonoids, glycosides, alkaloids, phenolic compounds and volatile constituents have been reported as the major phyto-constituents of the *Passiflora* species. Many *C*-glycosylflavones like orientin, isoorientin and schaftoside have been isolated from this genus. Their pharmacological effect against cardiovascular system and central nervous system has been investigated earlier. According to this view, a qualitative reversed phase high performance liquid chromatographic (RP-HPLC) method for the analysis of flavonoids in *Passiflora nepalensis* has been developed. This allows the isocratic separation of the major *C*-glycosylflavones occurring in this plant.

Keywords: *Passiflora nepalensis*, RP-HPLC, Isocratic elution, *C*-glycosylflavones

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 genus was used widely in traditional medicine in East India, Mexico, Netherland, South America, Italia and Argentina. One species of this genus named as *Passiflora nepalensis* (Passifloraceae) is more popular than its other species in Eastern India. *Passiflora nepalensis* is used in folklore medicine for treatment of CVD i.e. hypertension and inflammation[3-4]. Its medicinal usage has been reported in the traditional systems of medicine such as Ayurveda, Siddha and Unani. Antihypertensive and negative chronotropic effects of *Passiflora nepalensis* 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 renal ischemia/reperfusion has been established. The antioxidant property of apigenin-8-C-β-D-glucopyranoside, more commonly known as vitexin, has been reported in this genus[2-5]. *Passiflora* contains several compounds including alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds[2-4]. Many *C*-glycosylflavones like orientin, isoorientin and schaftoside have been isolated from this genus. Their pharmacological effects against cardiovascular system and central nervous system have been investigated earlier. According to these view, a qualitative reversed-phase high-performance liquid chromatographic method for the analysis of flavonoids in *Passiflora nepalensis* has been developed. This allows the isocratic separation of the major C-glycosylflavones occurring in this plant.

Material and Methods

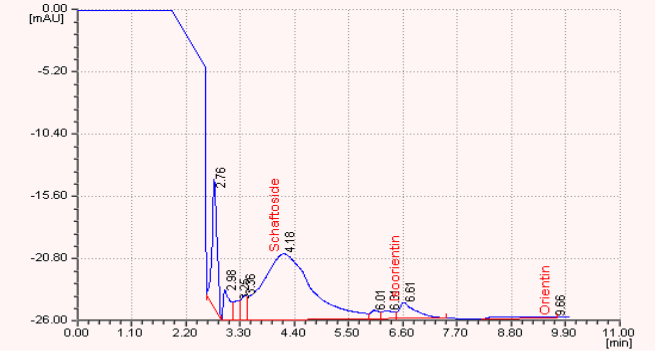
Plant material:
The whole plant of *Passiflora nepalensis* were collected in the month of October from the Eastern part of India (Sikkim Himalayas). The Herbarium specimen (No. 168) of plant was deposited in the Department of Pharmacognosy and it has been identified from Himalayan Pharmacy Institute, Majhitar. The whole plant was dried in shade and powdered (no. 60 mesh) and 3.5274 oz of the dried powder was soxhlet extracted successively with petroleum ether, benzene, chloroform, acetone, ethyl acetate and methanol. The weight of methanolic extract after drying was calculated as 0.1683 oz.

Isolation method:
The methanol extract was dark green residue were chromatographed over 200 g of silica gel (100-200 mesh) using petroleum ether, benzene, chloroform, ethyl acetate, acetone, methanol water and their mixtures in various proportion in the order of their increasing polarity (Table 1).

Preparation of the Column:
A glass column of 3.14 cm diameter was packed with activated silica gel in a form of slurry with hexane. The column was packed to height of 25 cm in order to establish a column volume of 150 ml. The column was built up by passing two column volumes of petroleum ether before the residue was loaded. The solvent was kept 5 cm above the bed and the residue was carefully loaded in the form of petroleum ether slurry. The column was then developed with a series of solvent starting with benzene, dichloromethane, chloroform, acetone, ethyl acetate and methanol in increasing polarity. The different ratio with succeeding solvents were fixed and fractions of 100ml were collected up to methanol-water system and thereafter, fractions in smaller volumes were collected, concentrated and checked with phytochemical analysis processed further.

RP-HPLC:
The HPLC analysis was performed using a LC-100, Cyberlab™, Salo Torrace, Millburry, MAO 1527, USA with LC-UV-100 UV detector. A CAPCELL (C-18) HPLC-packed column (4.6 mm I.D.X 250 mm), type MG 5 µm, number AKAD/05245 was used for the chromatographic separations. The mobile phase consisted of solvent [methanol: water (9:1)]. The separation was performed using isocratic elution (0-15 min) with a flow rate of 1.00 ml/min and a column temperature of 25.0°C. The injection volume was 25µl, and UV detection was effected at 254 nm. HPLC grades solvent were obtained from Shyam brothers, 27 sindhi market, Bhopal. After phytochemical analysis the (9:1) methanol: water extract (10µg/ml) were subjected to HPLC column and the obtained record were superimposed on the standard retention time values[6].

Fig. 1. RP-HPLC of methanolic extract of *Passiflora nepalensis* and retention time of their constituents



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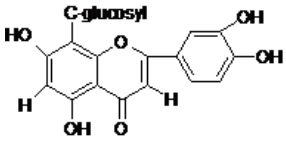
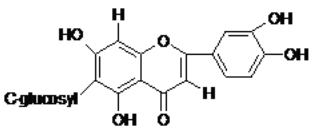
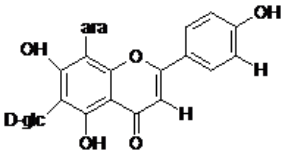


Orientin



Isoorientin



Schaftoside

Results and discussion

The construction of chromatographic fingerprints plays an important role in the quality control of complex herbal medicines[7]. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the "chemical integrities" of the herbal medicines and therefore be used for authentication and identification of the herbal products. Based on the concept of phyto-equivalence, the chromatographic fingerprints of herbal medicines could be utilized for addressing the problem of quality control of herbal medicines[8]. By definition, a chromatographic fingerprint of an herbal medicine is, in practice, a chromatographic pattern of pharmacologically active and or chemically characteristic constituents present in the extract. This chromatographic profile should be featured by the fundamental attributions of "integrity" and "fuzziness" or "sameness" and "differences" so as to chemically represent the herbal medicines investigated. This suggest that chromatographic fingerprint can successfully demonstrate both "sameness" and "differences" between various samples and the authentication and identification of herbal medicines can be accurately conducted even if the number and/or concentration of chemically characteristic constituents are not very similar in different samples of herbal medicine. Thus chromatographic fingerprint should be considered to evaluate the quality of herbal medicines globally considering multiple constituents present in the herbal medicines[9]. The methanolic extract analyzed displayed were reported on the figure 1, components and a number of peaks were superimposed on the standard extract at same conditions for retention time[6]. On the basis of these profiles, three major C-gycosylflavones were fractionated from methanolic extract of *Passiflora nepalensis* by open column chromatography over C-18 silica gel, subsequently separated by preparative HPLC and isolated in the form of light yellowish green amorphous powders soluble in methanol: water (9:1).

Conclusion

The therapeutic efficacy of the genus *Passiflora* extensively used in Indian System of Medicine has been established through modern testing and evaluation (pre-clinical and clinical trials) in different disease conditions. These studies place this indigenous drug a novel candidate for bioprospection and drug development for the treatment of such diseases as anxiety, insomnia, convulsion, sexual dysfunction, cough, cancer, postmenopausal syndrome, hypertension etc. The medicinal applications of these plants, countless possibilities for investigation still remain in relatively newer areas of its function. Hence, phytochemicals and minerals of these plants will enable to exploit its therapeutic use. Therefore further studies may carry out to prove the potential of these plants. This species is becoming the endangered species now so more work can be done on agricultural and climatic conditions to grow this plant.

Acknowledgment

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Table 1: Column Chromatography of methanolic extract of *Passiflora nepalensis*

Sl. No.	Date	Solvent system	Ratio	Conical flask no.	Total	Color of the residue
1.	09.3.09	Hexane	10 (1000)	1-10	10	Colorless
2.	10.3.09	Hexane: Benzene	8:2 (800+200)	11-21	10	Pale yellow
3.	11.3.09	Hexane: Benzene	5:5 (500+500)	22-32	10	Pale yellow
4.	12.3.09	Hexane: Benzene	3:7 (300+700)	33-43	10	Yellowish green
5.	12.3.09	Hexane: Benzene	1:9 (100+900)	44-54	10	Dark yellowish green
6.	13.3.09	Benzene	10 (1000)	55-65	10	Dark yellowish green
7.	14.3.09	Benzene: Chloroform	9:1 (900+100)	66-76	10	Dark green
8.	14.3.09	Benzene: Chloroform	8:2 (800+200)	77-87	10	Yellowish green
9.	15.3.09	Benzene: Chloroform	7:3 (700+300)	88-98	10	Yellowish green
10.	16.3.09	Benzene: Chloroform	5:5 (500+500)	99-109	10	Light yellow
11.	17.3.09	Benzene: Chloroform	3:7 (300+700)	110-120	10	Light yellow
12.	18.3.09	Benzene: Chloroform	1:9 (100+900)	121-131	10	Dark yellow
13.	20.3.09	Chloroform	10 (1000)	132-142	10	Yellowish brown
14.	21.3.09	Chloroform: Acetone	9:1 (900+100)	143-153	10	Yellowish brown
15.	23.3.09	Chloroform: Acetone	8:2 (800+200)	154-164	10	Yellowish green
16.	24.3.09	Chloroform: Acetone	7:3 (700+300)	165-175	10	Yellowish green
17.	24.3.09	Chloroform: Acetone	5:5 (500+500)	176-186	10	Yellowish green
18.	25.3.09	Chloroform: Acetone	3:7 (300+700)	187-207	20	Yellowish green
19.	29.3.09	Chloroform: Acetone	2:8 (200+800)	208-218	10	Yellowish green
20.	30.3.09	Chloroform: Acetone	1:9 (100+900)	219-239	10	Yellowish green
21.	16.4.09	Acetone	10 (1000)	240-250	10	Yellowish green
22.	17.4.09	Acetone: Ethyl acetate	8:2 (800+200)	251-261	10	Yellowish green
23.	19.4.09	Acetone: Ethyl acetate	7:3 (700+300)	262-272	10	Yellowish green
24.	20.4.09	Acetone: Ethyl acetate	5:5 (500+500)	273-283	10	Yellowish green
25.	21.4.09	Acetone: Ethyl acetate	3:7 (300+700)	284-294	10	Yellowish green
26.	22.4.09	Acetone: Ethyl acetate	1:9 (100+900)	295-305	10	Yellowish green
27.	24.4.09	Ethyl acetate	10 (1000)	306-326	20	Yellowish green
28.	26.4.09	Ethyl acetate: Methanol	9:1 (900+100)	327-367	40	Light yellowish powder
29.	01.5.09	Ethyl acetate: Methanol	8:2 (800+200)	368-398	30	Reddish brown
30.	05.5.09	Ethyl acetate: Methanol	7:3 (700+300)	399-419	20	Reddish brown
31.	07.5.09	Ethyl acetate: Methanol	5:5 (500+500)	420-440	20	Light yellowish powder
32.	08.5.09	Ethyl acetate: Methanol	3:7 (300+700)	441-461	20	Reddish brown powder
33.	09.5.09	Ethyl acetate: Methanol	1:9 (100+900)	462-527	65	Reddish brown
34.	18.5.09	Methanol	10 (1000)	528-553	25	Reddish brown
35.	20.5.09	Methanol: Water	9:1 (800+200)	554-579	25	Yellowish green
36.	21.5.09	Methanol: Water	8:2 (500+500)	580-600	20	Yellowish green
37.	23.5.09	Methanol: Water	7:3 (300+700)	601-621	20	Yellowish green
38.	25.5.09	Methanol: Water	5:5 (100+900)	622-642	20	Yellowish green