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Isolation and Identification of 5-O-Caffeoyl Quinic Acid from Industrial Herbal Residues of *Valeriana wallichii*

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In this work, the herbal residue obtained from plant parts of *Valeriana wallichii* was selected to evaluate the presence of bioactive compounds. The study included preliminary phytochemical analysis for detection of phyto-groups, column chromatography on ethyl acetate extract for isolation of possible phenolic compounds, and identification of isolated active compound by spectroscopic techniques. The phyto-chemical analysis showed presence of compounds such as phenols, flavonoids, terpenoids, glycosides and phytosterols. Spectroscopic analysis for active isolated compound indicated presence of a phenolic acid, 5-O-caffeoyl quinic acid, in the residues of *Valeriana wallichii*.

Keywords: *Valeriana wallichii*, 5-O-Caffeoyl quinic acid, Antioxidant.

INTRODUCTION

Herbal and Ayurvedic pharmaceutics consume large volumes of different medicinal herbs, of the order of a few hundred tonnes per annum, for production of various medicines [1]. Post production, the main by-products are herbal remnants including those from water extraction processes [2]. Since plants contain various groups of compounds having different polarities and solubility properties, the extraction processes may not remove significant volumes of active phytochemicals from the residues into their solutions for utilization in medicine manufacture. Preliminary studies conducted on different samples of these residues revealed presence of different bio active compounds [3]. No works have so far been reported on nature of industrial herbal residues with respect to possibility for recovery of bioactive compounds from them.

Valeriana wallichii, commonly known as Indian valerian and traditionally used as ingredient in numerous herbal medicines [4], is a small perennial herb belonging to family *valerianaceae*. *Valeriana wallichii* has natural habitats spread at high altitudes across the Himalayan ranges over India, Pakistan and South-Western China [5,6]. *Valeriana wallichii* is believed to have wide pharmacological end-use as analgesic, anti-inflammatory, hypotensive, antioxidant, antiviral and anxiolytic. Traditional medicines from *Valeriana wallichii* are also applied for health conditions such as bone and joint pain, carminative, digestive and sleep disorders, nervous and menstrual disorders, migraine, hysteria, neurosis and eye trouble [7,8]. Phytochemical studies in *Valeriana wallichii* roots report the presence of volatile oils, phenols, flavonoids, glycosides, sterols, iridoids and terpenoids [9,10].

This article describes a study undertaken to explore the nature of residue in the form of water extracted root of the herb *Valeriana wallichii* obtained from herbal pharmaceutical industry. The herb is considered a highly potent and endangered species of medicinal plant [11].

EXPERIMENTAL

The extracted *Valeriana wallichii* (EVW) roots were collected from a local pharmaceutical industry where the *Valeriana wallichii* was subjected to aqueous extraction process thrice. Physico-chemical and phytochemical analysis were carried out on washed, dried and coarsely powdered samples of extracted *Valeriana wallichii*.

Physico-chemical analysis: Physico-chemical parameters like loss on drying, ash content, acid insoluble ash content and extractive values were determined by standard methods.

Preparation of extracts: The dry powder was extracted successively with solvents such as hexane, ethyl acetate and ethanol using Soxhlet apparatus. The extractive values were determined after removing the solvents and compared against corresponding extractive values for fresh herb samples.

Preliminary phytochemical analysis: Preliminary phyto-chemical analyses were carried out on each extract for deter-

mining the presence of active phyto groups [12]. Presence of phenols were detected using phosphomolybdic acid and alcoholic ferric chloride, flavonoids with lead acetate and shinoda tests, tannins by Breamer's reagent, steroids and terpenoids using Leiberman-Burchard reagent. Presence of alkaloids were tested using Dragondorff's, Wagner's, Mayer's and Hager's reagents and phytosterols by Salkowski's Test and Liebermann-Burchard's test.

Estimation of total phenolic content: Total phenolic content was determined using Folin-Ciocalteu's reagent. The ethyl acetate and ethanol extracts of *Valeriana wallichii* appropriately diluted made up to 3.5 mL with distilled water in a series of test tubes. These tubes were then treated with 0.5 mL 2 N Folin-ciocalteu's reagent and incubated for 3 min at room temperature. The reaction was then neutralized by the addition of 1 mL of 20 % sodium carbonate. The reaction mixture was then incubated at room temperature for 90 min after which the absorbance was noted at 760 nm (Shimadzu UV-Visible Spectrophotometer, Model: 1800) and the percentage phenolic content is calculated from the graph plotted for standard gallic acid.

Isolation of phenolic compound: Ethyl acetate extract, which contain medium polar compounds were separated by column chromatography. The extract was eluted through wet filled stationary phase of silica gel column using toluene:ethyl acetate:ethanol (5:4:1) solvent system. 5 mL of fractions were collected and pooled according to thin layer chromatography (TLC) results. The fractions that answered positive to phenolic test were concentrated and further subjected to column chromatographic separation. The brown mass thus yielded were dissolved in ethyl acetate and purified by preparative TLC. The solvent system used was ethyl acetate: ethanol (1:1) which showed a dark blue spot at R_f of 0.47 when sprayed with 3 % sulphuric acid reagent. This was separated and identified by different spectral studies including UV-visible, FTIR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. UV-visible spectral analysis was carried out on UV-visible spectrophotometer (Shimadzu/1800) using ethanol as solvent. FTIR sampling was done by mixing finely powdered sample with KBr and pressed into a transparent disc of about 13 mm diameter and 0.3 mm thickness to run on FTIR instrument (Perkin Elmer/Spectrum One) over a range of 4000 to 450 cm^{-1} with 4.0 cm^{-1} resolution. NMR spectra were recorded using NMR spectrometer (Bruker/AVANCE III 500 MHz). Methanol-D_4 was used as solvent and chemical shifts were read relative to standard TMS. Further structural elucidation was studied by mass spectrometry using electron spray ionization method.

RESULTS AND DISCUSSION

Physico-chemical analysis performed on the extracted *Valeriana wallichii* yielded substantial extractive values for hexane, ethyl acetate, and ethanol extracts as shown in Table-1. The results indicate that extractive values for extracted *Valeriana wallichii* are of the order of 32 % of those obtained from fresh *Valeriana wallichii*. The results indicate the presence of significant amount of active metabolites in *Valeriana wallichii* herbal residues. The results of preliminary phytochemical analyses identifies the presence of different bioactive groups such as terpenoids and phytosterols (in hexane extract), phenols and flavonoids (in ethyl acetate) and phenols, flavonoids, steroids (in ethanol extract).

TABLE-1
PHYSICO-CHEMICAL ANALYSIS RESULTS OF *Valeriana wallichii*

Properties	Extracted	
	Valeriana wallichii	*Valeriana wallichii*
Loss on drying (%)	5.68	6.070
Ash (%)	2.08	5.798
Acid Insoluble ash (%)	0.76	1.078
Hexane extractive value (%)	1.008 ± 0.14	3.22 ± 0.55
Ethyl acetate extractive value (%)	0.985 ± 0.18	1.52 ± 0.84
Ethanol extractive value (%)	12.676 ± 1.18	33.928 ± 1.06
TPC in ethyl acetate extract (%)	3.236 ± 0.36	8.236 ± 0.48
TPC in ethanol extract (%)	8.13 ± 0.50	18.678 ± 0.36

In FTIR spectrum, a broad band was observed at 3402 cm^{-1}, characteristic of hydrogen bond of dimeric form of acids. Further, a weak band at 3785 cm^{-1} was observed indicative of terminal -OH group and a characteristic –CH=CH– was observed at 2161 cm^{-1}. >C=O group stretching vibration characteristic of organic acids was obtained at 1646 cm^{-1}. Bands at 1452-1039 cm^{-1} were observed suggestive of scissoring and ring stretching vibrations of cyclohexane. A band indicative of bending vibrations for CH_2 at 1445 cm^{-1} and a broad band at 718 cm^{-1} for aromatic OH group were also observed [13-15]. The UV-visible spectrum of isolated compound displayed λ_{max} (ethanol) at 325 nm. The NMR spectroscopy results are tabulated in Table-2. The ^{1}H NMR (CD_3OD) 500 MHz TMS signal were observed at δ (ppm) of 2.016, 2.21, 3.70, 4.17 and 2.071 representing different cyclohexane protons; with shielded proton signals at 6.24 and 7.56 indicating presence of *trans*-disubstituted ethylene moiety in the molecule. A broad singlet at δ 7.029 ppm represents trisubstituted aromatic ring. Carboxylic proton signal was observed as a singlet at δ 10.1 indicating presence of COOH group in the compound. ^{13}C NMR spectrum showed presence of sixteen carbon atoms. Two carbonyl group signals were observed at δ 176.0 and 167.20. Signals at δ 36.76(C6), 37.33(C2), 70.51(C3), 72.06(C4), 70.57(C5) and 74.71(C1) represent different cyclohexane carbons. Six aromatic carbon signals appeared at δ 113.86(C2'), 115.07(C5'),

TABLE-2
^{13}C NMR AND ^{1}H NMR CHEMICAL SHIFTS

Number of carbon	^{13}C NMR	^{1}H NMR
1	74.71	–
2	37.33	2.01t
3	70.51	2.21d
4	72.06	3.70t
5	70.57	4.17s
6	36.76	2.07d
7	175.61	–
1'	126.36	7.02s
2'	112.86	–
3'	143.33	–
4'	148.11	–
5'	115.07	6.94 d
6'	121.58	6.75d
7'	145.66	7.56d
8'	113.79	6.22d
9'	167.27	–

121.58(C6'), 126.36(C1'), 145.33(C3') and 148.11(C4'). Signals at δ 145.66 and δ 113.80 correspond to olefinic carbons at positions (C7') and (C8'). The ^{1}H NMR and ^{13}C NMR spectroscopic data are comparable with those of 5-O-caffeoyl quinic acid reported by several workers [16-18].

With reference to mass spectrum (Fig. 1), molecular ion peak $[M+H]^+$ at *m/z* 355 in positive ion mode and base peak at *m/z* 163, may be inferred as loss of quinic acid moiety [19,20]. From these spectroscopic data and literature concordance, the isolated compound is identified as 5-O-caffoeyl quinic acid, commonly known as chlorogenic acid, with molecular formula $C_{16}H_{18}O_9$ [21-23]. Fig. 2 shows structure of the isolated compound.

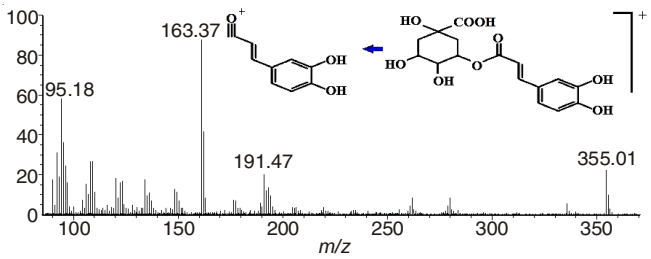

Fig. 1. Mass spectrum of isolated compound

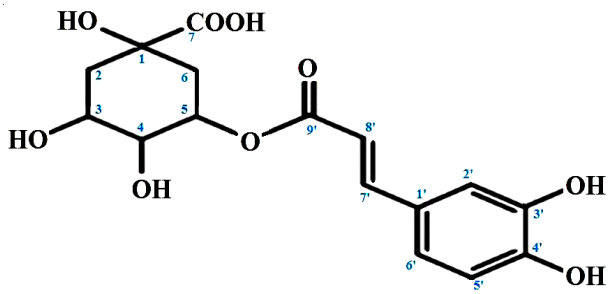

Fig. 2. Structure of 5-O-caffeoyl quinic acid

Structurally, 5-O-caffeoyl quinic acid is an ester formed between caffeic acid and quinic acid and is an important biosynthetic intermediate in lignin biosynthesis. It is widely found in many plant species and the presence in different valerians including *Valeriana wallichii* [24-26]. It is a biologically and pharmaceutically important natural phenolic compound. It is a strong antioxidant and neuroprotective agent [27,28]. Nakajima *et al.* [29] reported that they can inhibit the oxidative stress induced neurotoxicity *via* antioxidant actions. Ito *et al.* [30] reported that chlorogenic acid and its *in vivo* metabolites, *m*-coumaric acid increased spontaneous loco motor activity in mice and was found effective for promoting neuronal differentiation.

Conclusion

The present study explores the fact that herbal residues generated in Ayurvedic medicine manufacturing industries containing many pharmaceutically important compounds. The study resulted in the isolation and identification of a potent antioxidant phenolic acid, 5-O-caffeoyl quinic acid from the industrial remnants of *Valeriana wallichii* roots, thus by indicating scope of their utilization for recovering biologically active compounds before discarding. This study also identifies a new, economical and environment friendly source for a multi-functional natural anti-oxidant.

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