Chromatographic Separation Studies of Bioactive Compound from *Artemisia Nilagirica* (Clarke) Pamp

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Abstract

In recent years, traditional system of medicine has become a topic of global importance wherein the plant metabolites plays an important role as source of pharmaceuticals for various treatments. Herbal medicines are termed as 'Alternate medicine' and their of great demand in both developed and developing countries for their wide biological medicinal activity, safety and lesser cost. WHO estimated 80% showed positive correlation between modern therapeutics and traditional herbal medicines. *Artemisia nilagirica* (clarke) pamp is a sweet-smelling herbaceous perpetual plant has a place with Asteraceae family was usually known as Sage Brush or wormwood and they were developed for its religious significance because of its huge scent.

In the present investigation, the leaf extract of *Artemisia nilagirica* (clarke) pamp was subjected to column chromatographic separation and the fractions were subjected to thin layer chromatography. The Rf value was authenticated to be 0.71 for flavonoids from *Artemisia nilagirica*. The fractioned compound was subjected to HPLC and the peak was absorbed in the range of 6.623 which represented the compound to be flavonoid.

Keywords: Flavonoids, Bioactive compounds, chromatographic techniques, medicinal plant, HPLC.

I. INTRODUCTION

The use and popularity of traditional medicines are gaining importance throughout the world enabling the drug discovery for safe and less cost drugs [1]. Many bioactive compounds Viz; Volatile oils, Flavonoids, Terpinoids, Tannins, Steroids, Proteins, Artemesinin, Exiguaflavone A and B, Macckianin and 2- (2, 4- hydroxyl phenyl) -5, 6- were reported from *Artemisia nilagirica* [2, 3, 4]. Natural products and related drugs are used to treat 87% of all categorized human diseases including cancer, bacterial infection and immunological disorders. About 25% of the prescribed drugs has been originated from plants source about 80% of the population in developing countries relies on traditional plant- based medicines for their primary health care needs [5]. *Artemisia nilagirica*used to determine bioactive natural products in the form of antimicrobial proteins /peptides, and the may serve for the development of new drugs [6, 7]. In traditional medicine, Protein and peptide were used in different disease status like cancer, diabetes, immune modulating, neuro degenerative effects as drug targeting ligands to the specific site [8]. Literature survey revealedmany methods for analysis of carboristeine in plasma and bulk drugs by Fluorimetric, Spectrophometric, and High Performance Liquid Chromatography [9]. An attempt has been made to develop a new precise and accurate HPTLC method for estimation of carbocisteine in the information as per ICH guidelines [10]. Clear FDA and ICH guidelines are followed by the pharmaceutical industries for characterization (both physical and chemical) and licensing etc [11]. Various parameters such as degradation mechanisms, potential degradation and degradation of products, pathways and interactions of the drug and determination of excipients in drug products includes stability testing of a new drug production [12]. Ion exchange chromatography, RP-HPLC methods are reported to be the best methods for confirming the purity and the characteristics of a bioactive compounds [13]. The bioactive constituents or phytochemicals are proved to be highly potential drugs after various prediction of lesd molecules and validation of molecule by précised analysis [14]. In the present investigation, the isolated and purified flavonoid compound from *Artemisia nilagirica (Clarke) Pamp*was authenticated by HPLC method.

II. MATERIALS AND METHODS

Genus *Artemisia* (Asteraceae) popularly known as sage brush or wormwood is a bitter aromatics. The whole plant was air dried after segregation as leaves, stem, flowers and roots and made in to power and stored in air tight container for further study.

A. Column Chromatography

The crude methanolic leafextract (20g) was mixed (adsorbed) with silica gel (100-200 mesh) (Merck) and chromatographed on a silica gel initially eluting with continuous suitable solvent system and gradually increasing the polarity mixture of solvent (Hexane, Hexane: Chloroform, Chloroform, Chloroform: Ethyl acetate, Ethyl acetate, Ethyl acetate: Ethanol). The fractions were eluted using TLC and similar TLC pattern were pooled in to major fraction to obtain (126) a pale greenish amorphous powder. 20 grams of the Sample were chromatographed over silica gel column (100 – 200 mesh). The mixture was packed on a silica gel column (Merck, India) and eluted with 100% hexane and then solvents were added in the increasing order of their polarity namely Chloroform,Ethyl acetate and Ethanol in the ratios of 90:10, 80:20,70:30, 60:40 and 50:50. Based on TLC profile, the eluates were pooled into some fractions. Column fraction126 (Ethyl acetate:Ethanol – 50:50) gave a solid which was crystallized from methanol to yield a pale greenish amorphous powder (Yield: 125mg).

B. Thin Layer Chromatography

Thin-layer chromatography (TLC) is the simplest and cheapest method of detecting plant constituents [15]. The thin layer chromatography was developed in twin through chamber with silica gel 60 F254 Pre coated aluminium plate of 0.2 mm thicknes using ethyl acetate: methanol (1:1) as the developing solvent system. Rf values were calculated and visualized by dipping the plate in vanillin sulphuric acid (1%) and heated on 105^0 C and the color of the spot appeared distinctly under visible light, short UN 245 nm and long UV 365 nm.

C. High Performance Liquid Chromatography (HPLC):

Instrumentation - Ginkgo HPLC Program (Ginkgo.m) with HP Chemstation software system. HPLC column 150x3.2mm, Column temperature was maintained at 35^o C. Mobile phase- Mixture of solvent A (0.1% Phosphoric acid solution) and solvent B (Methanol) 50:50, Flow rate, 1.2Ml/min/Injection volume was 10*M*L. Detection wavelength was 270nm and running time was 40 minutes. DAD detector option: Diode array Detector data were acquired on a mode 1100 series DAD detector over the wavelength range 200-400nm at a rate of 1 pectrum/second and 1.2nm resolution.

III. RESULTS

Thecrude extract (20 g) was subjected to separation of bioactive compounds by column chromatography on silica gel (100-200 Mesh -Merck). About 289 fractions were separated out and these fraction were subjected to Thin layer chromatography. During the present investigation, the Rf values were calculated by measuring the movement of the solvent and the solute. About 289 fractions were identified under visible range short UV 245 nm and long UV 365 nm. The isolated fraction Quercetin was observed at the 121th- 133rd fraction of separation with the Rf value of 0.71. The purified bioactive compound quercetin was subjected to High Performance Liquid Chromatography (HPLC) for authentication of the compounds. The sample was injected through the HPLC column and registered four different peaks and the results are tabulated in the Table 1 and the chromatogram of the identified compound is given in the Fig .1. The isolated compound was subjected to quantification studies with different concentration and the results are tabulated in the Table 2.

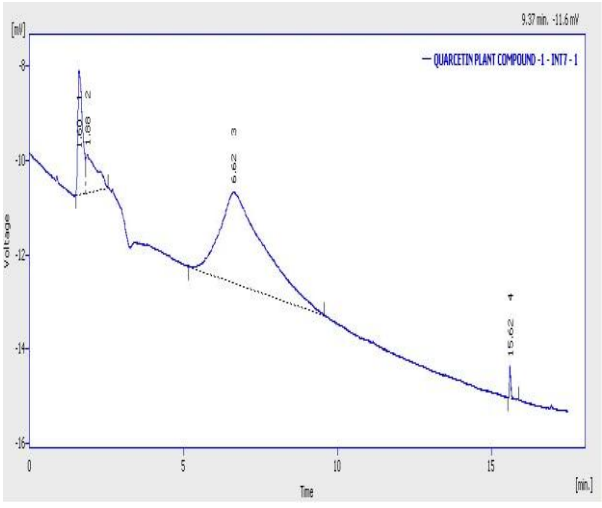

Fig 1: HPLC Chromatogram of isolated Quercetin from leaf extract of *Artemisia nilagirica (Clarke) Pamp*

Table 1: HPLC results of Quercetin

S.No	Reten. Tim (min)	Area (Mv.s)	Height (Mv)	Area (%)	Height (%)	WOS
1.	1.603	26.642	2.567	13.5	45.7	0.18
2.	1.880	7.026	0.566	3.6	10.1	0.30
3.	6.623	161.124	1.807	81.9	32.2	1.26
4.	15.620	1.871	0.676	1.0	12.0	0.04
	Total	196.666	5.617	100.0	100.0	

Table 2: Quantification results of purified Quercetin.

S.No	Concentration (mg)	Area in millivolt	Quantification of Quercetin (mg)
1.	0.25	166.06	0.0285
		161.12	0.0276
2.	0.025	58.33	0.0247
		56.61	0.0240
3.	0.1	16.67	0.0253
		16.65	0.0252

IV. DISCUSSION

Separation of bioactive constituents of *Artemisia nilagirica (Clarke) Pamp*was carried out by column chromatography and the different fractions are further they were subjected to Thin layer chromatography. About 289 fractions were isolated from the leaf extract of *Artemisia nilagirica (Clarke) Pamp*and the quercetin was found to be between 121-133 fractions [16]. Methanolic leaf extractof *Polyathia longifolia*has been reported to have 20 known and 2 unknown phytochemicals which was highly cytotoxic [17]. From the present TLC investigation, it was observed that the Rf value of quercetin was reflected in 0.71 and it was also authenticated [18].In the present investigation the isolated bioactive compound quercetin was separated validated using column chromatography and High Performance Liquid Chromatography (HPLC) to ensure specific accurate compound. The results of HPLC like retention time and the peak area determines the relationship of the concentration of quercetin in the sample [19]. The HPLC analysis of the *Artemisia nilagirica*leaf revealed flavonols, quinines, essential oils, lectins, tannins, polyohenols, phenolics, terpenoids, polypeptides [20].

V. DISCLOSURE STATEMENT

The phytochemical of the medicinal plants are referred to contain high therapeutic value for its low cost and less side effects. From the present investigation *Artemisia nilagirica (Clarke) Pamp*has proved to have high medicinal value in terms of bioactive compounds isolation and it also proved to contain quercetin which proved to cure many human ailments.

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