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ISOLATION AND CHARACTERIZATION OF BETA SITOSTEROL FROM ROOT OF *ECHINACEA PURPUREA*

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Abstract

Echinacea purpurea (EP) is one of the most important medicinal plants belonging to the family Asteraceae. The plant is perennial and native of North America and widely distributed in eastern, south eastern and mid western USA and Canada but not grown in India due to climatic changes. The plant is well known for immunostimulant activity which is due to active constituents present in the plant. Many compounds were isolated and established their chemical structure. In the present study, hydro-alcoholic extract of the roots of the plant was subjected to partition chromatography to separate out constituents at various fractions. The chloroform, water-acetone insoluble and water-acetone soluble fractions were subjected to column chromatography to isolate and purify the phytoconstituents to get markers. β Sitosterol was isolated and purified from Fraction 1 (chloroform Fraction). The identified compound was subjected to spectral studies *viz.,* NMR, MASS and IR followed by HPLC method for clear separation and quantification of the same by standardized the mobile phase and other chromatographic conditions. The present investigation was aimed to isolate and characterization of new flavonoid containing compound from the root extract of *Echinacea purpurea* (EP).

Key words : *Echinacea purpurea*, β sitosterol, Phytoconstituents, HPLC.

Introduction

About 70,000 plant based drugs are used medicinally in world wide. Among that traditional system of medicine especially Ayurvedic system of medicine uses 2000 plants to cure different health ailments. It is estimated that about 80% of the world population depends on herbal based alternative system of medicine (Ayurveda, Unani medicine, & Chinese traditional medicine) due to its safe application, less expensive than synthetic medicine and less side effect. Therefore, herbal medicine is now gaining focus in the world market in ethno medicinal practices and new drug discovery(Mukherjee PK *et al.,* 2002) A medicinal herb produces and contains a number of secondary metabolites which are responsible for biological activities as well as important therapeutic efficacy(Anonymous *et al.,* 2003). A plant or plant based product are generally used as food and preparations of other beverages which provides health benefits as nutrient sources but alternatively a large variety of other chemicals that serves as the important functions which are relatively unknown(Daniel M *et al.*,2006).

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Echinacea purpurea (EP) is the perennial plant belongs to the family Asteraceae, has a high demand in the economic World market. Though the plant is native of The United States of America and Canada but also is cultivated as medicinal plant in parts of north and eastern Africa and in Europe(Seemannova Z *et al.,* 2006) and also in the hilly area of India. The plant is widely used medicinal plants in America, Europe which is mainly used to treat upper respiratory tract infection, immunostimulant (Thomson *et al.,* 2012) antioxidant, wound healing (D Barsett L *et al.,* 2005, Lee TT *et al.,* 2009). Traditionally the root and subterranean stem were used by North America in early period to treat trauma and alleviate symptoms of infection and inflammation. These activities are mainly due to the presence of active constituents. It was revealed EP contains mainly caffeic acid derivatives,

alkamides (Gajalakshmi S *et al.,* 2012), flavonoids, essential oils, polyacetylenes. Thereafter, few more constituents were also isolated *viz.,* alkamides, three ketoalkenes, two ketoalkynes, and four phenolic acids (cichoric acid, caftaric acid, and chlorogenic acid) etc from 70% aqueous ethanolic extract.(Gajalakshmi S *et al.,* 2012) Caffeic acid derivatives and alkamides shows immunoregulation effects and antioxidant activity. EP root was subjected for extraction, fractionation, isolation for new compounds in which alkamide fraction, polysaccharide containing fractions gave caffeic acid derivatives which resulted pharmacological activities (Brown D *et al.,* 2005). *EP* is currently of considerable interest due to immunomodulatory (Lazarowych N J *et al.,* 1998), anti-inflammatory, antiviral and cannabinoidreceptor 2 (CB2) binding activities (Fatma AM *et al.,* 2017) of its alkylamide constituents. Alkylamides were later recognized as insecticidal and oncolytic, cyclooxygenase-I (COX-I) and cyclooxygenase-II (COX-II) inhibitory activity (Brown D *et al.,* 2005). Based on this concept, the present study was undertaken to isolate new active compounds from the roots of EP and characterization of the same through NMR, MASS and IR studies for drug discovery.

Materials and Methods

Collection of Plant Material

Dried powder extract (1 kg) of *EP* (Lot no-EP100-120 201) was procured from Natural Remedies Private Limited, Bangalore and further study was carried out.

Extraction and Fractionation

Preparation of column

180g of chloroform Fraction was dissolved in minimum quantity of 100% chloroform and adsorbed over silica gel (# 60 – 120) in the ratio 1:2 (drug to silica gel) and finally dried under vacuum below 60^0 C. A big column of 10 litres capacity was first loaded with 1 kg of silica gel (# 60-120) with chloroform as solvent (dry packing).180g of charging material was charged and the elution was started with pet ether and increasing the polarity with methanol. The Fractions collected were concentrated by distillation under vacuum in a rota vapour.

Fractionation of the extract

The hydro-alcoholic extract was dissolved in mixture of chloroform and water. The chloroform part was separated and water part was again partitioned with acetone to separate out the different solvent soluble constituents. The schematic representation for partition of hydro-alcoholic extract is shown in flow chart.

Pre-column preparation

A glass column was selected and rinsed with acetone. A cotton layer was placed at the bottom and chloroform was poured into the column. Stationary phase was then added to the column in a ratio of 1:6 (drug: silica gel) to make stationary bed for separation of constituents. The column was left for few hours for complete saturation and air bubbles were removed. The dried charging material was charged into the column. Another layer of cotton was placed over the charged material to prevent the disturbance of the extract bed while pouring mobile phase. The charged column was eluted with different mobile phases with gradual increase or decrease in polarity.

Collection of fractions

The solvent used for elution in column were collected as various fractions. They were concentrated and dried in rotavapour (Buchi R-114) and were subjected for thin layer chromatographic studies

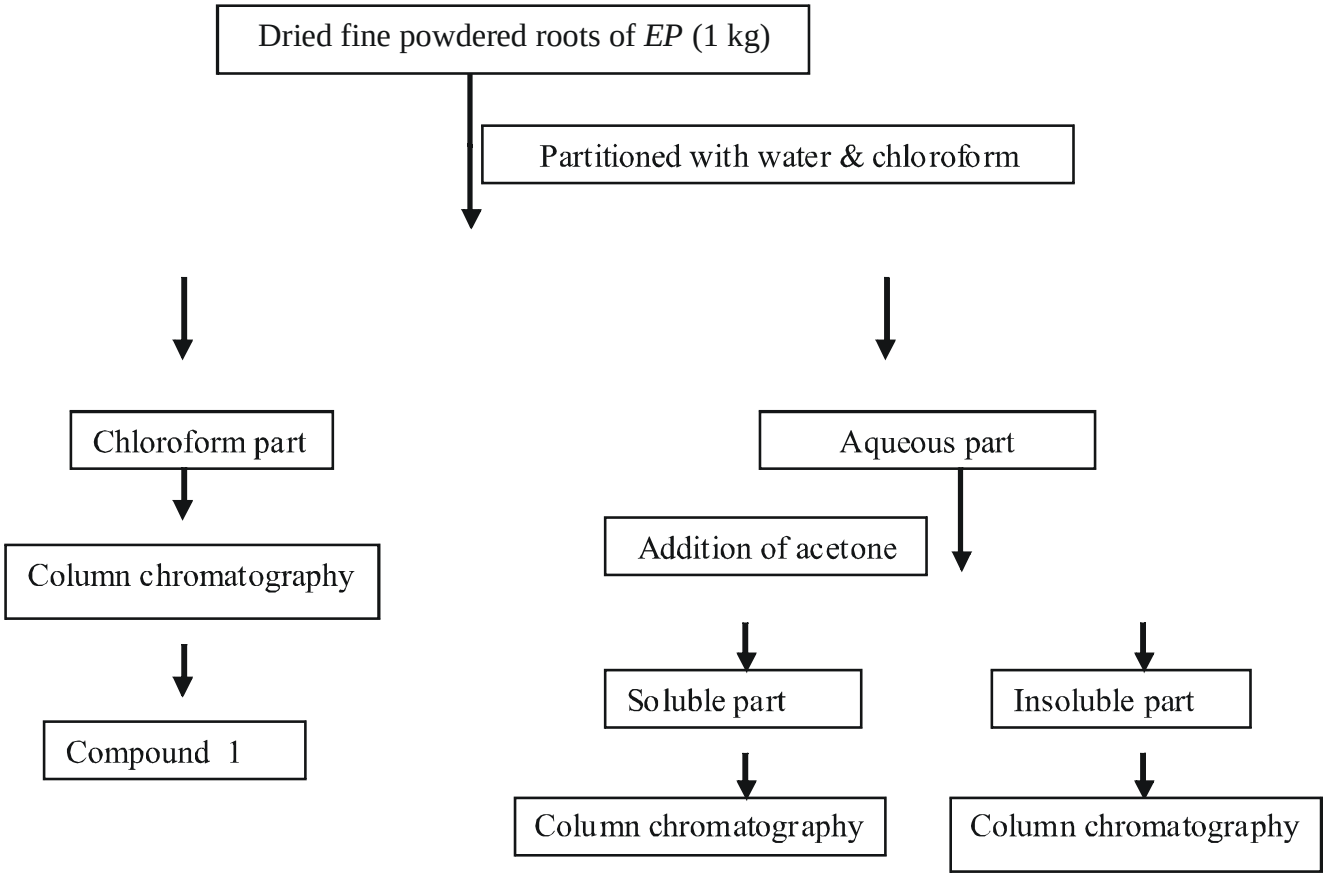
Purification of Fraction-7 and 8 of column

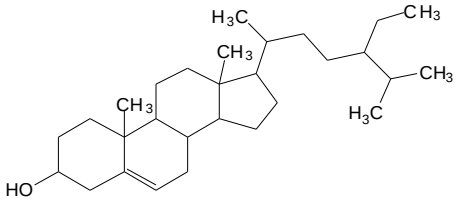
Fraction 7 and 8 mixed and was kept in acetone and as a result compound 1 was precipitated out as a pure compound which was further identified by using TLC. 10% MeOH in chloroform Fraction (Fraction 7) and 15% MeOH in Chloroform (fraction 8) showed single spot on TLC plate by ANS with less impurity. Initially little impurity was present with the compound which was further purified by preparative HPLC The purity of the compound was analyzed by Analytical HPLC. The characterization of the compound was done by Melting point, FTIR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopy respectively. The compound is identified as β sitosterol.

Results and Discussion

The isolated portion from chloroform Fraction of the *EP* was chromatographic over silica gel to afford beta sitosterol. The presence of the compound beta sitosterol was established by using IR, NMR and MASS spectroscopy. Rf value (0.06) was obtained and melting point 148-149ºC uncorrected which coincided with the standard. It showed a distinct peak of Rt time 14.250 with an area of 2620655 and 97% purity. The 1H NMR spectra of beta sitosterol exhibited a signal corresponding to the proton connected to C3 hydroxyl group which appeared as multiple at δ3.52 (1H,m).Six other proton signals were evident which include four secondary methyl group δH 0.92, 0.85, 0.825 and 0.80 all doublets. Two tertiary methyl group δH 0.68 and 0.93. In IR analysis absorption bands appeared at 3580-3200 indicates the presence of OH group. Peak at 2870 indicates C-H

Flow chart 1: Enrichment process





Analytical HPLC:

Column	**KROMOCIL (C-18 (250*4.6*7µ)**		
Flow rate	1 ml/ min		
Detection	205nm		
Instrument	Shimadzu		
Mobile phase	Pump A (136mg KH_2PO_4 + 0.5ml H_3PO_4 + HPLC water q.s. to 1000ml) Pump B (methanol)		
Gradient time program	Time	A Conc.	B Conc.
	0.01	95	5
	30	95	5
	30.01	stop	-
Injection volume	20µl/injection		
Detector	Photodiode array detector		

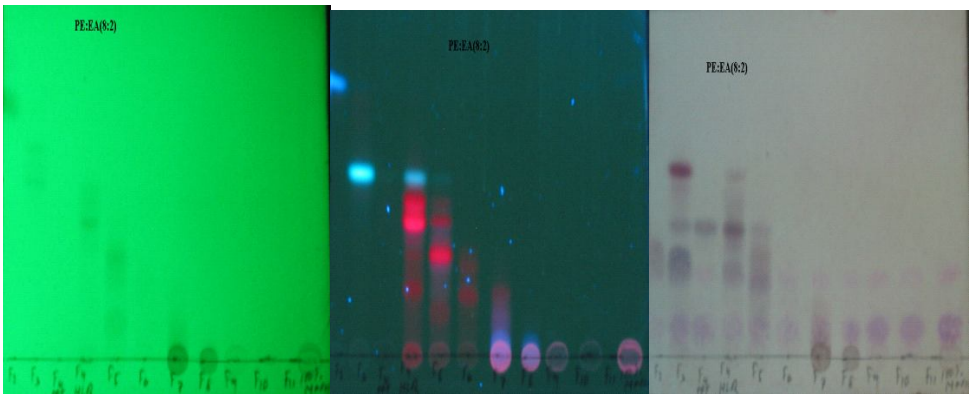


Fig. 1: TLC profile of column 1.

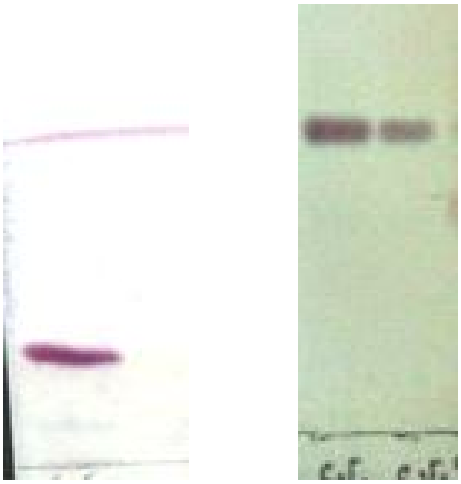


Fig. 2: Fraction 7 and 8 was mixed and TLC was carried out. The spot was visible only after ANS.

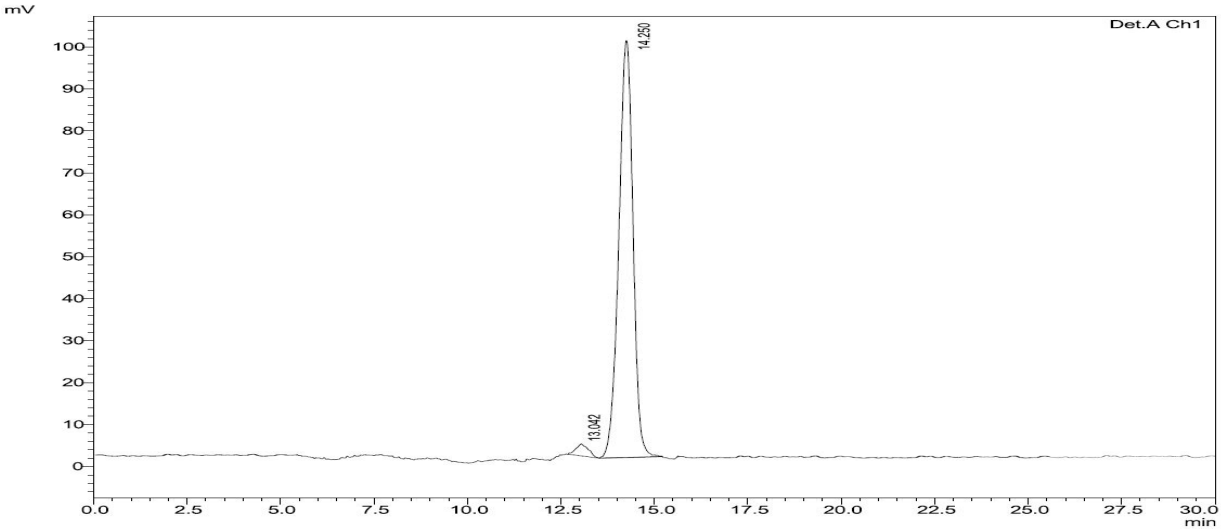


Fig. 3: HPLC Chromatogram.

stretching, 1582 peak indicates C=C stretching, 1016 peak indicates C=O.

Conclusion

The simplicity of isolation and analysis of the compound suggest that the compound may be termed as marker for standardization, and its cost effective method compared to all other existing isolating method.

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