Bioguided extraction and characterization of various fractions of *Dichrostachys cinerea* (L.) Wight. and Arn. Roots

Rajesh Bolleddu, Anasuya Datta, Kalyan Hazra, Sreya Dutta, Jyoti Dahiya, Anupam K Mangal

Department of Pharmacognosy, Central Ayurveda Research Institute, CCRAS, Ministry of AYUSH, Kolkata, West Bengal, India, 700091

Abstract

An Ayurvedic plant, *D. cinerea- Veerataru* known for its medicinal value and used traditionally in the treatment of diabetes, rheumatism, strangury etc., The shade dried root powder of *D. cinerea* was extracted with 80% aqueous ethyl alcohol by maceration process. After maceration period the contents are filtered and filtrate was concentrated under reduced pressure by rotary flash evaporator. The concentrated ethanolic extract of roots, further fractionated with petroleum ether, chloroform, ethyl acetate and butanol. The resultant fractionated extract is concentrated under reduced pressure. Phyto pharmacological studies revealed that among all extracts ethyl acetate and butanol extracts are rich source of phenols, flavonoids and having considerable antidiabetic activity. Qualitative HPLC studies of ethyl acetate and butanol extracts revealed the presence of catechin in ethyl acetate extract and rutin in butanol extract respectively.

Introduction

- *Dichrostachys cinerea* Wight. and Arn. belongs to Mimosaceae family is a small tree, occur in Northwestern, Central and Southern India.
- In Ayurveda, *D. cinerea* plant is used as *asmari, trsna, mutrakrcchra, mutraghata and sandhisula*. Roots are used in asthma, diuretic conditions. It possess nephroprote ctive and antibacterial activities.

The methanolic extract of root significantly protected mice against the Russell's viper venom. Diuretic property observed for Kwatha of these roots. Root bark reported to possess mild anticonvulsant activity. β-sitosterol, β-amyrin, and n-octacosanol were isolated from benzene extracts.

- Ethyl acetate and butanol fractions reported to possess highest antioxidant & anti diabetic properties than all other extracts of *Dichrostachys cinerea* roots.
- Hence the present study aimed for phytochemical investigation through High- performance liquid chromatography (HPLC) analysis to know the the presence of active phytoconstitutents which are responsible for therapeutic activity.

Materials and methods

Preparation of ethanolic extract

Root powder (2.5 kg) was macerated with 80% ethyl alcohol (8 days). The solvent was separa -ted from the extract in a rotavapor and dried.

Fractionation of the mother extract

To the 100 g of d ethanolic extract 500 ml of distilled water were added and fractioned with petroleum ether, chloroform, ethyl acetate, and butanol. The solvent was separated from the extract in a rotavapor & dried.

HPLC Analysis

Ethyl acetate & butanol fractions were qualita -tively tested with standard biomarkers. HPLC analysis was carried out on the Waters HPLC Autosampler model 717 plus, operated with the Empower2 software. The mobile phase was being 5% glacial acetic acid in water and acetonitrile for Catechine (40:60%) and 5% Glacial acetic acid in water and methanol for Rutin (70:30% v/v). Detection was carried out at 360nm (rutin) and 279nm (catechin). The ethyl acetate fraction, butanol fraction and standard catechin, rutin samples were dissolved in methanol. The standard concentration sample, i.e., 10 ppm, was injected into the HPLC system. Injection volume is 20µl, run time is 12 min with a flow rate of 1 ml /min.

Discussion

- The *D. cinerea* roots were dried, grounded and extracted with 80% aqueous ethyl alcohol by cold maceration and fractionated with various organic solvents viz., petroleum ether, chloroform, ethyl acetate and n-butanol.
- The extracts were subjected to qualitative phytochemical investigations and analytical studies.
- The phytochemical screening of *D. cinerea* extracts given positive results for phenols, flavonoids. Quantification of phenols, flavo -noids revealed that ethyl acetate and butanol extracts of *D. cinerea* root are rich in phenolic, flavonoid compounds among all extracts.
- In HPLC analysis, the *D. cinerea* root ethyl acetate extract chromatogram shown a well resolved peak at the retention time of 5.036min which is almost coinciding with standard Catechin retention time of 5.017. Similarly in butanol extract of *D. cinerea* root chromatogram a well resolved peak was appeared at the retention time of 5.168min which is almost identical to standard Rutin eluted at mean retention time of 5.157 min. These results confirming the presence of Catechin in ethyl acetate extract, Rutin in butanol extracts of *D. cinerea* root

Results

- ✓ The yield of aqueous ethanolic extract of *D. cinerea* root is 120 g. The percentage yield of aqueous ethanolic extract is 5% and petroleum ether, chloroform, ethyl acetate, n-butanol, & left over extracts were 1.8, 4, 12, 30, and 30%.
- ✓ Total phenolic content of ethyl acetate, butanolic extract is 158mg GAE/gm, 80mg GAE/gm
- ✓ Total flavonoid content of ethyl acetate, butanolic extract is 32 mg RE/gm, 24 mg RE/gm

HPLC analysis: Catechin eluted at mean retention of 5.017 min. Rutin eluted at mean retention of 5.157 min. The retention times of Catechin in ethyl acetate extract was 5.036 and the retention time of Rutin present in the butanol extract was found to be 5.168 respectively which are identical to standard reference compounds. Figure 01 to 04 represent HPLC chromatograms for standard catechin, rutin and test extracts of *D. cinerea,* respectively.

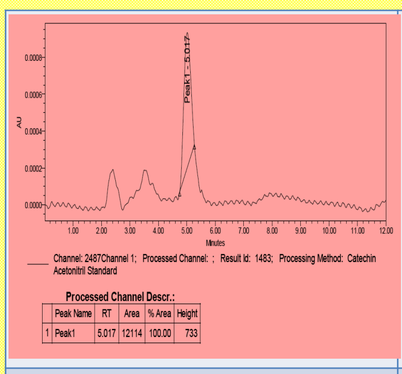

Fig. 1: HPLC chromatogram of Catechin (Standard)

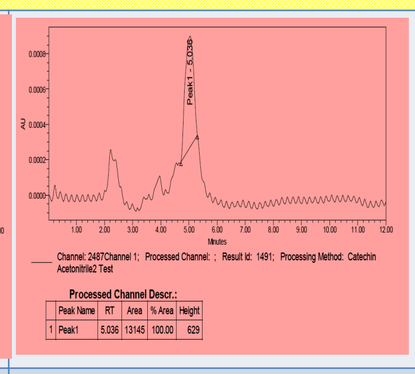

Fig. 2: HPLC chromatogram of Ethyl acetate fraction

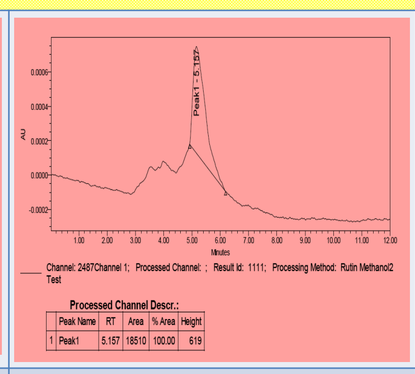

Fig. 3: HPLC chromatogram of Rutin (Standard)

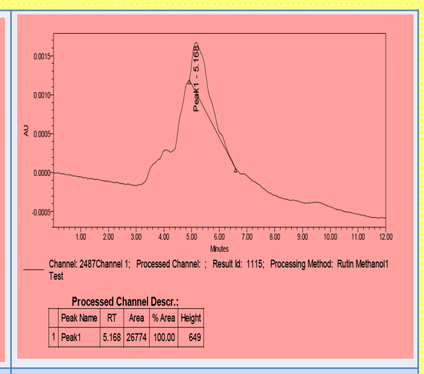

Fig. 4: HPLC chromatogram of Butanol fraction

Conclusion

- Traditionally *D. cinerea* plant is claimed to be useful in the treatment of diabetes.
- The claims made for this plant in traditional systems in the treatment of diabetes are proved to be correct based on characterization of antidiabetic biomarkers.
- Fractionation of 80% aqueous ethanol extract proven to be successful for proper characterization of active phytochemicals.
- The HPLC analysis of ethyl acetate, butanol extracts of *D. cinerea* showed that catechin was the active phytoconstituent identified in the ethyl acetate extract followed by rutin in butanol extract.

References

- Bolleddu R, Venkatesh S, Rao MM, Shyamsunder R. (2019) Investigation of the pharmacognostical, phytochemical, and antioxidant studies of various fractions of *Dichrostachys cinerea* root. J Nat Sci Med; 2:141-6. DOI: 10.4103/JNSM.JNSM_56_18.
- Bolleddu R, Venkatesh S, Hazra K, Rao MM,Shyamsunder R. Anatomical and antihyperglycemic activity of *Dichrostachys cinerea* roots. Med J DY Patil Vidyapeeth 2020;13:258-63.
- Vijay L, Bhatia AK, Anjana G, *et al*., (2015) Phytochemicals Screening and Analysis using HPLC to determine the Antimicrobial efficacy of *Cassia fistula* extract. *Adv Biores*. 6(3), pp.1-7.