Asian Journal of Chemistry; Vol. 33, No. 12 (2021), 3099-3102

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23455

HPLC Fingerprinting of Chloroform Extracts of Seven Ethnomedicinal Plants of Mizoram, India

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Received: 2 September 2021; *Accepted*: 12 October 2021; *Published online*: 6 December 2021; AJC-20604

The present work was done for seven ethnomedicinal plants used by the inhabitants of Mizoram in order to investigate the presence of various phytoconstituents. The root-stock of *Alocasia indica*, leaves of *Bidens pillosa*, *Chromolaena odorata*, *Elaeagnus caudata* and *Spilanthes acmella*, the latex of *Carica papaya* and rhizomes of *Curcuma caesia* were dried and powdered. The chloroform extract of each sample were prepared by soaking dried powdered samples in chloroform for 72 h. The extracts were filtered using Whatman filter paper No. 42 (125 mm). The filtrates of plant extracts were preserved at 4-5 ºC for further process. Crude extracts of selected plants parts were analyzed using TLC coupled to HPLC fingerprinting, which gives some prominent and moderate peaks with different retention time, which may be a bioactive compounds.

Keywords: Ethnomedicinal plants, Phytoconstituents, TLC, HPLC fingerprinting, Retention time.

INTRODUCTION

Ethnomedicine involves the incorporation of knowledge, beliefs, values, skills, health, clinical and non-clinical practices of society members related to human health [1]. According to WHO, in developing countries for health care requirements, approximately 80% population mainly relies on natural or plant-based medicines [2,3]. WHO defines traditional medicine as "diverse health practices, approaches, knowledge and exercise applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent human illness". With the changes in traditional culture and arrival of modern medicines, the traditional indigenous knowledge about medicinal plants of different ethnic groups, which was orally passed down from a generation to other for centuries, was forgotten. Thus, ethno-medicinal information must be properly documented for its preservation [4].

For development of novel drugs, phytochemical studies conducted on the basis of ethno-pharmacological information are effective [5]. Flavonoids, alkaloids, phenolic compounds, and tannins are some of the most useful and valuable bioactive plant compounds. Phytochemical studies of plants have shown the existence of different phytochemicals such as alkaloids, terpenoids, flavonoids, steroids, saponins, phlobatannins, tannins and cardiac glycosides. Previously, brief TLC profiling of these plants were also performed [6].

HPLC analytical method is used for drug analysis and isolation of natural products. This chromatographic technique is gaining popularity among several analytical techniques as the main option for fingerprinting study for the quality control of herbal plants [7]. Fingerprint analysis has been accepted by WHO as a methodology for authentication and identification of herbal medicines [8,9]. HPLC technique helps to identify closely related plant species, to detect adulterations, to purify the individual components of the mixture and to detect stability of the same extract over time.

Mizoram is a north-eastern (NE) state in India and one of global 25 hotspots of mega-biodiversity [10]. More than 400 medicinal plant are species found in Mizoram state of India. Local population uses these plants for healthcare purposes. *Alocasia indica, Bidens pillosa, Carica papaya, Chromolaena odorata, Curcuma caesia, Elaeagnus caudata* and *Spilanthes acmella* are some plants, which are widely utilized as herbal medicines. Hence, this study conducted HPLC fingerprinting

on the specified plants and provided scientific support for their traditional use.

EXPERIMENTAL

Collection of plant materials: *Alocasia indica* (Roxb.) Schott, *Bidens pillosa* L., *Carica papaya* Linn. and *Curcuma caesia* were collected from Mizoram University campus whereas *Chromolaena odorata* (L.) R.M. King & H. Rob., *Elaeagnus caudata* Schlecht. exMomiy and *Spilanthes acmella* Murr. were collected from Champhai district of India. All the plants samples were identified by the elders of the Mizo community and authenticated by the Department of Horticulture Aromatic and Medicinal Plants, Mizoram University, Aizawl, India.

Extraction of plant materials: Non-infected root-stock of *Alocasia indica*, leaves of *Bidens pillosa*, *Chromolaena odorata*, *Elaeagnus caudata* and *Spilanthes acmella*, the latex of *Carica papaya* and rhizomes of *Curcuma caesia*, were air-dried, grounded, powdered and extracted by maceration in chloroform at room temperature for 72 h. The individual extracts were filtered using a Whatmann filter paper No. 42 (125 mm) and concentrated using a rotary evaporator. The filtrates of plant extracts were preserved at 4-5 ºC for further process.

Thin layer chromatography (TLC): TLC profile of various plants were performed on a sheet of aluminium foil which is coated with a thin layer of adsorbent silica gel, which are commercially available 60 F_{254} (Merck). The plant extracts were spotted in 1 mm diameter above the bottom of the plate and placed into mobile phase. The extracts were allowed to move on the adsorbent (stationary) phase according to the solvent system used. Several combinations of solvents of increasing polarity were evaluated as mobile phase in TLC to determine the number of compounds present in all these plants extracts. The TLC plates were kept in a solvent mixture of *n*-hexane:ethyl acetate (7:3), air dried sprayed with Dragendorff's reagent and the spots were visualized in day light and under UV light. The ratio of the distance moved by the solute (compound) and the distance moved by the solvent is defined as R_f value. The formation of reddish brown precipitate indicated the presence of alkaloids. In all cases, same TLC plate and solvent system was used [11,12].

High pressure liquid chromatography (HPLC): The high-pressure liquid chromatographic system consists Waters 515 HPLC pump, a valve type injector, Waters 2489 UV/visible detector (Water, Singapore), Symmetry® C_{18} (250 mm × 4.6 mm) column with a particle size of 5 µm. Acetonitrile, water (HPLC grade, E. Merck, India) in the ratio 70:30 were used for the analysis. Other conditions: temperature-ambient, flow rate-0.5 mL/min, injection volume-5 µL, detection at 254 nm.

RESULTS AND DISCUSSION

The plants selected for the HPLC fingerprinting are used by the traditional healers, their medicine uses are listed in Table-1.

TLC: The evaluations of various plants extract showed presence of different components as indicated by a varying number of spots on a TLC plate. The R_f value of various components are indicated in Table-1.

HPLC: The present investigation was carried out on a few important plants, which are medicinally used by the practioners of traditional healers. TLC and HPLC fingerprinting were carried out to substantiate their traditional use. The results obtained can be used for the genuine identification of the plants.

The qualitative HPLC fingerprint profiles were selected at a wavelength of 254 nm due to sharpness of the peaks and

TABLE-1
TLC SOLVENT SYSTEM FOR PLANTS EXTRACTS WITH R_f VALUES AND USES

Plant name	Parts used	Solvent system	Spot identification method	R_f value	Uses	Ref.
Alocasia indica	Root stock	*n*-Hexane/ ethylacetate (7:3)	Under sunlight, UV 254 nm. Sprayed with Dragendorff's reagent gives Intense orange spot	0.2, 0.3 0.51,0.55 0.67,0.70	Antifungal Anti-inflammatory	[13-16]
Bidens pillosa	Leaves	*n*-Hexane/ ethylacetate (7:3)	Under sunlight, UV 254 nm. Sprayed with Dragendorff's reagent gives Intense orange spot	0.2, 0.24 0.54,0.61 0.69,0.85	Anti-inflammatory, anti-diabetic Anticancer and antitumor.	[17-19]
Carica papaya	Latex	*n*-Hexane/ ethylacetate (7:3)	Under sunlight, UV 254 nm. Sprayed with Dragendorff's reagent gives Intense orange spot	0.2,0.37 0.48, 0.57 0.71	Curing jaundice, diabetes, food poisoning and dog bites	[16]
Chromolaena odorata	Leaves	*n*-Hexane/ ethylacetate (7:3)	Under sunlight, UV 254 nm. Sprayed with Dragendorff's reagent gives Intense orange spot	0.15, 0.33 0.52, 0.60 0.74, 0.85	Anti- diarrheal, astringent, antihypertensive Anti-inflammatory.	[16,20]
Cucurma caesia	Rhizomes	*n*-Hexane/ ethylacetate (7:3)	Under sunlight, UV 254 nm. Sprayed with Dragendorff's reagent gives Intense orange spot	0.16, 0.29 0.54, 0.75	Dysentery, jaundice, asthma, measles and food allergy	[16]
Elaeagnus caudate	Leaves	*n*-Hexane/ ethylacetate (7:3)	Under sunlight, UV 254 nm. Sprayed with Dragendorff's reagent gives Intense orange spot	0.57, 1.3 1.7, 1.9 2.7, 3.9	Used for expelling placenta and stop the menses Health tonic.	[16,21]
Spilanthes acmella	Leaves	*n*-Hexane/ ethylacetate (7:3)	Under sunlight, UV 254 nm. Sprayed with Dragendorff's reagent gives Intense orange spot	0.14, 0.31 0.44, 0.51 0.65	Curing paralysis of the tongue, toothache and a popular remedy for children who are stammer Anti-inflammatory and analgesic.	[16,22]

proper baseline. The results of HPLC analysis (Fig. 1a) of *Alocaesia indica* chloroform extract, at 254 nm, shows presence of various constituents as evidenced by the chromatogram obtained at various retention times (2.915, 3,599, 4.114, 5.271, 6.073, 6.814 and 7.243) are the constituents found in *Alocaesia indica* rhizome mainly. Fig. 1b shows the chromatogram of the chloroform extract of *Bidens pillosa* leaves at 254 nm. This wavelength was selected because the major 6 peaks showed a maximum absorption at this wavelength. The eluted compounds were detected in the range of 1-15 min at retention times (2.803, 3.995, 5.211, 6.155, 6.797 and 10.953). The chloroform extract of *Carica papaya* latex chromatogram (Fig. 1c) shows different constituents at various retention times (2.880, 4.006, 6.177, 6.889 and 7.346). These peaks represent the main constituents present in the plant latex. The eluted compounds were detected in the range of 2-15 min.

Fig. 1d shows the chromatogram of chloroform extract of *Chromolaena odorata* leaves with different peaks at various retention times (2.986, 4.110, 4.573, 6.231, 7.005, 7.477 and 8.241). The chromatogram of chloroform extract of *Curcuma caesia* rhizome (Fig. 1e) shows with two main peaks (eluted at 3 min and 6.3 min) and other six peaks showed a maximum absorption at this wavelength. The chloroform extract of *Elaeagnus caudata* leaves chromatogram (Fig. 1f) shows different constituents at various retention times (2.884, 3.990, 5.159 and 6.134). These peaks represent the main constituents present in the plant leaves. The eluted compounds were detected in the range of 2-15 min. Similarly in *Spilanthus acmella* (Fig. 1g) show the various constituents with different retention times (2.906, 6.199, 7.629 and 10.439), in which the compound having RT 2.906 is the main constituents in chloroform leaf extract. The eluted compounds were detected in the range of 2-12 min.

Conclusion

TLC profiling has shown the presence of various phytoconstituents in the selected plants of the present study. HPLC is highly useful for authentication, identification of herbal medicines, isolation and purification of the plant based products. Both TLC and HPLC techniques provide the chromatogram of different compounds present in chloroform extracts of seven ethnomedicinal plants (*Alocasia indica*, *Bidens pillosa*, *Carica papaya*, *Chromolaena odorata*, *Curcuma caesia*, *Elaeagnus caudata* and *Spilanthes acmella*) of Mizoram state of India. However, further biological fingerprinting need to be investigated. Therefore, these methods must be coupled to HPLC-MS, IR, UV and NMR analyses in order to identify the purity and quality of the compounds detected.

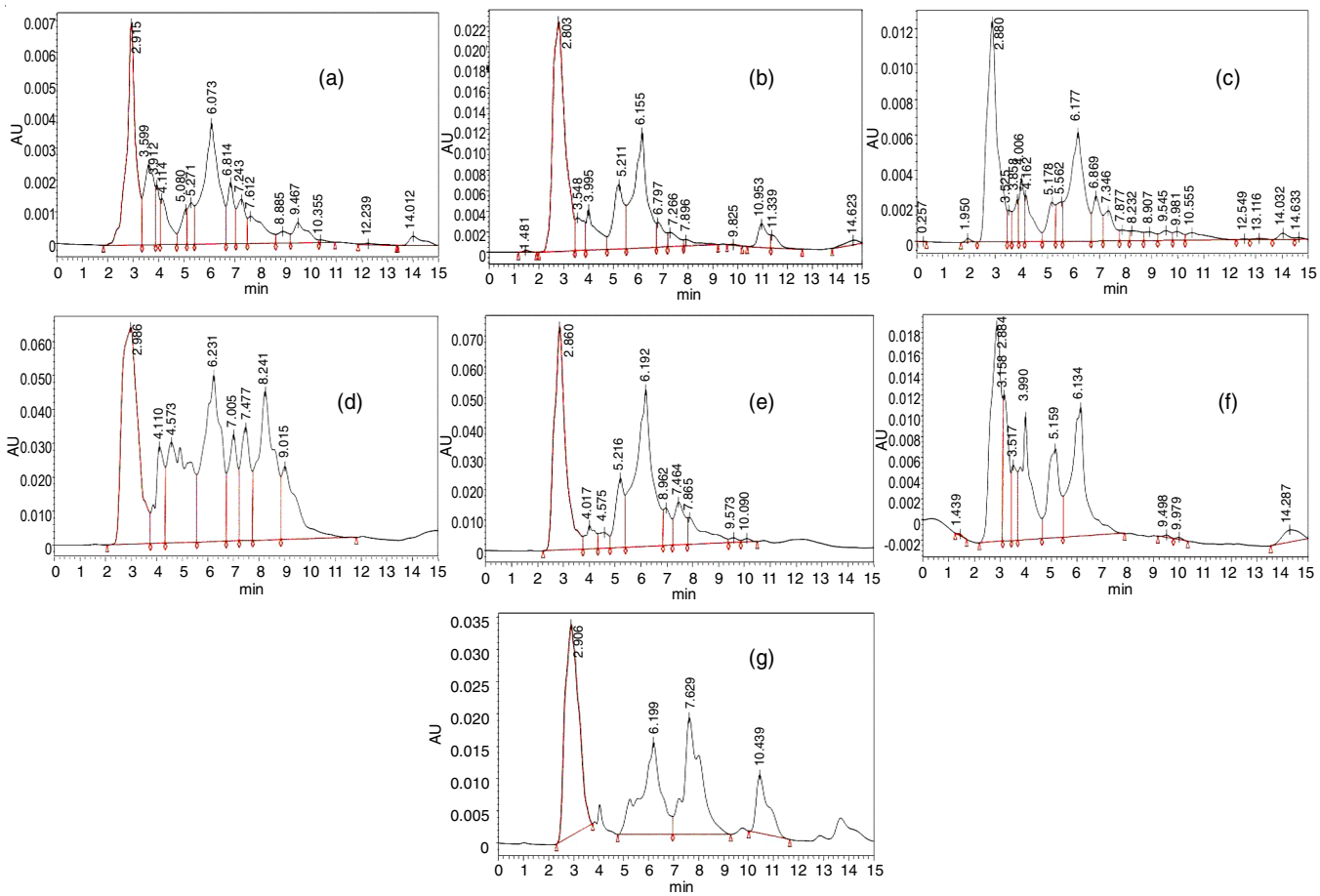

Fig. 1. HPLC chromatogram of crude chloroform extracts of (a) *Alocasia indica* Root-stock, (b) *Bidens pillosa* leaves, (c) *Carica papaya* latex, (d) *Chromolaena odorata* leaves, (e) *Curcuma caesia* rhizome, (f) *Elaeagnus caudata* leaves and (g) *Spilanthus acmella* leaves at 254 nm

ACKNOWLEDGEMENTS

The financial support from Non-SAP UGC Meritorious fellowship to Longjam Shantabi and Department of Biotechnology, Government of India, New Delhi vide Grant No. BT/60/NE/TBP/2011 to GCJ to carry out the study is gratefully acknowledged.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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