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Short Communication

Active constituents in *Artemisia vulgaris* L.

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Abstract

Artemisia vulgaris L is one of the oldest traditional medicines and used for treating different aliments. In recent years, many reports were published on its activities of active principles and pharmaceutical potentials. As a part of studies, the chemical profiling of *Artemisia vulgaris* L. has been investigated using the spectroscopy method running in the different gradients of mobile phase in the C18 Accucore RP MS 150 X 4.6 mm. Herein, the isolated compounds, such as Cynarin. L-Saccharopine, 13-Acetyl-9-dihydrobaccatin III, 4, 5-Dicaffeoylquinic acid, Quercetin -1′ –glucoside or Spireoside are reported.

The characterization of aqueous extract of traditional medicine is challenging due to the poor retention of the analytes on conventional C_{18} columns. This study presents a systematic characterization method based using C18 Accucore RP MS 150 X 4.6 mm column for aqueous extract of *Artemisia vulgaris* L. UHPLC-LCMSMS method was used to profile components in both untargeted and targeted manners by ITMS +PESI Full MS acquisition approach. The components were identified by fragmentation rules elucidation, reference standards and databases. This phytochemical inquiry of this work is beneficial to explore the economical and medicinal uses in future.

Keywords: *Artemisia vulgaris* L, Phytochemical profiling, Sikkim Himalaya.

INTRODUCTION

Artemisia vulgaris L. is the potent medicinal plant of the Eastern Himalaya predominantly occurs in the sub-temperate and temperate regions ranging from 1200-2500 meter. It is used for treating various ailments such as nose bleeding, nausea, dizziness, skin diseases and also high altitude sickness. Decoction prepared from the young shoot is used to increase appetite and promote digestion. Leaves are used to fumigate the houses to repel insects (Chhetri and Rai, 2018). Extract prepared from semi decomposed plant is used for the pest management in agriculture by the farmers in Sikkim. This plant also has cultural and religious significances among the indigenous communities of Sikkim (Pradhan and Singh, 2019). More than ten species of *Artemisia* are found in Sikkim and its adjoining regions. However, the chemical profiling of this plant has not been reported from this region. The present study provides the data on the availability of active compounds in *Artemisia vulgaris* L.

During the experiment, the study considered to isolate and characterize the active compounds using the different solvent mixtures. Although, in recent past, the studies on the *Artemisia vulgaris* L. conducted for the isolation and characterization by several authors such as Ekiert *et al.* (2020), Thien (2018), Gaudencio *et al.* (2011), Consolacion *et al.* (2008), Nikolova *et al.* (2004), Vetschera *et al.* (2003) etc. In line with, this experiment attempts to study on the availability of the active principles in *Artemisia vulgaris* L. in MeOH –Water fraction.

MATERIALS AND METHODS

Plant material

The specimen collected from vicinity of Gangtok (1700m), East Sikkim, India and identified in the Sikkim State Forest Herbarium (SSFH). Voucher specimens were deposited at the Sikkim State Forest Herbarium (SSFH) under the designation SSFH SK002530.

Preparation of crude methanolic extract and fractionation

The powdered crude drug (25 g, aerial part) was extracted twice over a period of 48 h by shaking with 400 ml MeOH and water at room temperature. The extract was evaporated almost to dryness and dissolved in 200 ml MeOH –H_2O (1:4). The MeOH –H_2O mixture was extracted with petroleum ether b.p 40-60°C (4 x 50 ml) (fraction a) and with EtOAc (5 x 50 ml) (fraction b). The remaining MeOH –H_2O extract resulted in fraction c. The fraction c was freeze dried and redissolved in 20 ml each of the solvent for the further study.

Analytical methods

Detector type: MS. Peak Detect: ICIS. Filter : ITMS +Pesi Full MS. Trace : TLC Mass Range.View Width (Min): 25.00. ICIS Peak integration. Smoothing Point 11. Baseline window 80. Area noise factor 2. Peak noise factor 10.
Column oven Temperature=30° C. Sample Temperature=10° C. %A Equate = 5mM, 0.1% Formic acid. Water. % B Equate = Methanol. %C Equate =Acetonitrile. % D = Methanol: Water(80:20). DrawSpeed: 10 µl /s. DrawDelayn= 200 [ms]. DispSpeed =20.00 µl/s. Waste speed =100 µl/s. WashVolume= 100 µl/s. Data Collection rate : 4.0 Hz. UV_VIS : 254 nm.
Autozero setting. Flow 0.4 ml/min. UV ready and column ready. UV-VIS acquisition on. Flow=0.4 ml/min. 0.5 Flow =0.4 ml/min. %B = 2.0 %, %C= 0% . %D= 0.0 %; 2.00. Flow= 0.4 ml/min %B = 40.0 %, %C= 0% . %D= 0.0 %. 20. Flow= 0.4 ml/min. %B = 95.0 %, %C= 0% . %D= 0.0 %; 22.00 Flow= 0.4 ml/min. %B = 95.0 %, %C= 0% . %D= 0.0 %;22.1 Flow= 0.4 ml/min. %B = 2.0 %, %C= 0% . %D= 0.0 %; 25.00 UV-VIS Acq. Off Flow= 0.4 ml/min. %B = 95.0 %, %C= 0%, %D= 0.0 %.

RESULTS

The solvent using CH_3OH and Chloroform chromatographed for the fractional isolation which was later treated with the gradients of solvent and water to obtain the target compound. The extract was chromatographed on a saphadex LH -20 Column (400 x 40 mm) and eluted by the different gradient of MeOH: Chloroform to give five fractions.

Compound I isolated and the mass spectrum indicated the molecular ion peak (M^+) at m/z 539.18 , RT 3.48. ; 19 m/z values (intensities) 215 (123) 216 (33.0) 265 (19), 267 (26), 299 (64), 300 (81), 304 (44), 314 (22), 331 (27), 359 (225), 360 (54), 377 (999.0), 378 (103), 392 (19), 454 (15), 478 (98), 479 (28), 493 (24), 521 (50) LCMSMS : Precursor m/z: 539.18.
Calcd for $C_{25}H_{24}O_{12}$ (M^+); Found: 356.1256. Compound: Cynarin.

Compound II isolated and the mass spectrum indicated the molecular ion peak (M^+) at m/z 274.39 , RT 23.18 ; 4 m/z values (intensities) 102 (72), 230 (64), 256 (999), 257 (311); LCMSMS : Precursor m/z: 274.39; Calcd for $C_{11}H_{20}N_2O_6$ (M^+); Found Mol. wt: 276. Compound: L-Saccharopine.

Compound III isolated which depicted the molecular ion peak (M^+) at m/z 371.14 , RT : 19.40; 3 m/z values (intensities) 311 (999) 329 (563), 355 (635); LCMSMS : Precursor m/z: 274.39; Calcd for $C_{38}H_{42}O_{12}$; Found Mol. wt: 630. Compound: 13-Acetyl-9-dihydrobaccatin III.

Compound IV isolated having the molecular ion peak (M^+) at m/z 499.18 , RT : 3.50; 12 m/z values (intensities): 145 (48); 163 (493 , 164 (82), 174 (41), 273 (44), 298 (51), 319 (999), 320 (111), 325 (74), 436 (65), 441 (48), 466 (44); LCMSMS : Precursor m/z: 499.18; Calcd for $C_{33}H_{12}O_{12}$; Found Mol. wt: 516. Compound: 4, 5-Dicaffeoylquinic acid.

Compound V isolated showing the molecular ion peak (M^+) at m/z 463, RT : 3.50; 26 m/z values (intensities): 107 (37.97), 121 (15.99), 151 (999), 152 (80.93), 176 (11.99), 179 (808), 182 (9.99), 192 (19.98), 193 (54.95), 194 (20.98), 211 (15.99), 217 (48.96), 228 (16.98), 229 (103.91), 233 (11.99), 239 (14.99), 240 (56.95), 246 (44.96), 257 (33.97), 258 (22.98), 259 (17.98), 272 (17.98), 273 (99.9), 283 (25.98), 300 (24.98), 310 (510.54); LCMSMS : Precursor m/z: 463; Calcd for $C_{21}H_{20}O_{12}$; Found Mol. wt: 464. Compound: Quercetin -1′ –glucoside or Spireoside.

DISCUSSION

The MeOH –H_2O (1:4) extract of *Artemisia vulgaris* L filtered passing through the $MgCl_2$ and NaCl for the removal of impurities. After the filtration, the sample extract chromatographed on a saphadex LH-20 column (400 x 40 mm) with the solvents gradients of %A Equate = 5mM, 0.1% Formic acid. Water. % B Equate = Methanol. %C Equate =Acetonitrile . % D = Methanol: Water (80:20) in MS^n Detector whereby the data collection rate is 4.0 Hz per second.
Interpreting the fragmentation patterns of the molecules, the results depicted the presence of L-saccharopine, 13-Acetyl-9-dihydrobaccatin III, 4,5 Dicaffeoyl-quinic acid, quercetin-1′ glucoside or spireoside and cyarin in *Artemisia vulgaris* L. Thus, the findings of the five novel compounds from the specimen enrich the database of *Artemisia vulgaris* L. but the efficacies of these compounds need some bioassay studies which can unravel the uses.

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