Chemistry of Natural Compounds, Vol. 40, No. 6, 2004

LYOPHILIZATION AND FREEZE-PRECIPITATION AS A METHOD FOR CRUDE EXTRACTION OF CATHINONE FROM *Catha edulis* LEAVES WITH MINIMUM THERMAL INJURY

Mehret Yerdaw Banjaw and Werner J. Schmidt UDC 547.945

Studies on *Catha edulis* illustrated the importance of using freshly harvested young shoots and leaves since the active component, cathinone, readily converts to a reduced product, cathine, upon drying and storage of the cut plant material [1–2]. Therefore, the aim of this study is to find a method that is simple but suitable for extraction of cathinone in the crude extract with minimum thermal injury.

Catha edulis leaves (3000 g) were collected from the Southern region of central Ethiopia. A voucher specimen was deposited at Addis Ababa University herbarium, Addis Ababa (voucher number 1340, Mehret Yerdaw Banjaw in Melaku Wondafresh). The fresh leaves were then immediately freeze-dried (–20°C) and transported to Germany with the permission of the German Government.

The methods are described below in detail. The crude extract of *Catha edulis* leaves was prepared by freeze-precipitation and lyophilization procedures. The methods yielded 0.35% (w/w) lyophilized crude extract. Its cathinone (5 mg/mg lyophilized extract) was determined using a spectrophotometer [3] and HPLC (peak area and retention time of cathinone in crude extract 1018.90 mAU*s & 3.1 min compared with standard cathinone: 7633.5 mAU*s & 3.028 min). A further verification was also made using TLC plates. Eluent mixture: ethyl acetate–methanol–ammonia, 85:10:5; spots were viewed in an Ultraviolet cabinet (254 & 366 nm) and sprayed later with ninhydrin solution.

All chemicals, reagents, and solvents were from Sigma–Aldrich Chemie GmbH Kappelweg1, Schnelldorf, Gemany. The freeze-dried plant was finely chopped, weighed, and placed in an Erlenmeyer flasks (400 g per flask) wrapped with aluminium foil in order to avoid light induced decomposition. Then reagent grade chloroform (150 ml) and diethyl ether (450 ml) in a 1:3 v/v ratio were added to cover the minced leaves (400 g). The resulting mixture was stirred using a shaker (Janke and Kunkei GmbH and Co.Ka IkA LaborTechnik Staufen 100/min) for 24 h under dark condition and 20°C. The content was later filtered through folded filters (604 ½, 150 mm diameter, Filtration Life Science, Schleicher and Schuell GmbH Daseel, Germany).The filtration made it easy to separate the cellulose material. The filtrate (450 ml) was again passed through a round filter (595, 90 mm, Filtration Life Science, Schleicher and Schuell GmbH Daseel, Germany) with the help a of mini filter pump. Fractions of a organic filtrates (400 ml) collected in this way were pooled together, kept in wide mouth amber bottles, and left at –20°C overnight. The freezing produced two distinct layers: a dark brown precipitate and green pigment-rich layers. Decantation separated the precipitate from the green pigment-rich layer. Then the trace organic solvent present in the precipitate was removed with the help of an air hood (3 h) and afterward subjected to the lyophilization procedure. The lyophylization was done using a freeze dryer (LYOVAC GT2, FINN-AQUA® GmbH, Hurth, Germany). In this procedure, the extract was first cooled to –30°C for 24 h in a separate deep-freezer and then dried in a freeze- drier for 24 h using a heating programmer that ranges from –30°C to 20°C. Primary drying: temperature ranging from –30° to 5°C (14 h) followed by secondary dryinget 20°C (10 h).

The spectrophotometer method was based on treating the reductant cathinone with copper (II) – neocuproine reagent in sodium acetate followed by measuring the absorbance [3]. Absorbance was measured using an Ultrospec3000, UV/Vis Spectrophotometer, Pharmacia Biotech, wavelength 455 nm and 1ml cuvette (OS). The content of the crude extract was determined using the linear regression equation ($y = mx + b$) of the standard commercial cathinone (absorbance vs. concentration) stock solution (1600 mg/ml). Standard cathinone was prepared as a stock solution of 1600 mg/ml for series

Zoological Institute, Department of Neuropharmacology, University of Tubingen, Auf der Morgenstelle 28E, 72076 Tubingen, Germany, Fax +49-7071-295144, e-mail: werner.schmidt@uni-tuebingen.de. Published in Khimiya Prirodnykh Soedinenii, No. 6, pp. 502-503, November-December, 2004. Original article submitted May 28, 2004.

0009-3130/04/4006-0611

dilution. The stock solution for lyophilized extract was also prepared by dissolving 100 mg in 100 ml distilled water. The standard stock solution was further diluted (1:25) with reagent and the absorbance measurement was made. Series dilutions were made for crude extract.

Thin layer chromatography (TLC) was done based on a previous report [1]. Stationary phase: 0.25 mm thick silica gel 60 G (Merck) glass plates. Eluent: acetate–methanol–ammonia (85:10:5). Spray reagents 0.3% (m/v) ninhydrin solution in a mixture of alcohol R + 3% (v/v) acetic acid.

HPLC Analysis. For the analysis we used a standard reverse-phase HP 1090 M system comprised of autosampler (Hewlett-Packard), integrator (HP3392 A), software (HPLC-3D-DOS Chemstation, Rev.02.02 Hewlett Packard), and reversed phase column (Nucleosil C18, 5 mm, 125 mm × 4.6 mm I.D.Grom). Spectral width: C18, 5 mm, 125 mm × 4.6 mm I.D.Grom). Spectral width: 200–600 nm. Separations were done in the gradient mode using solvent **1**: 0.1% phosphoric acid in H_2O. Solvent **2**: acetonitril: time (min) 0, **1)** [100%] and **2** [0%], 15 min: **1)** [0%] and **2)** [100%], 16 min: **1)** [100%] and **2)** [0%], flow rate 2 ml/min, injection volume: 10 ml, UV-Diod-Array-Detection (DAD) at 260 nm.

ACKNOWLEDGMENT

The scholarship grant by Katholischer Akademischer Auslander Dienst (KAAD) to the first author is gratefully acknowledged.

REFERENCES

1. K. Szendrei, *Bull Narc*., **32**, 5 (1980).
2. S. Geisshusler and R. Bresnneisen, *J. Ethnopharmacol,* **19**, 269 (1987).
3. A. M. Al-Obaid, S. A. Al-Tamrah, F. A. Aly, and A. A. Alwarthan, *J. Pharm. Biomed. Anal*., **17**, 321 (1998).