Khat plant: alkaloid extraction and identification

INTRODUCTION

Khat (Catha edulis), belongs to the suborder Rosidae and family Celastraceae. The main ingredients of khat leave include alkaloids, tannins, and flavonoids (Feyissa and Kelly, 2008). The major alkaloids in khat leaves are cathinone, cathine, and norephedrine (Feyissa and Kelly, 2008, Kalix, 1990). The average concentrations of cathinone, cathine, and norephedrine in fresh khat leave of various origins are 0. 95, 1. 98, and 0. 54 mg/g of khat leaf, respectively (Dimba et al., 2004, Feyissa and Kelly, 2008, Geisshüsler and Brenneisen, 1987, Widler et al., 1994). The major contents of adult khat leaves are cathine and norephedrine at a ratio of approximately 4: 1 (Kalix, 1990, Schorno and Steinegger, 1979). Cathine and norephedrine occur mainly in older plants and is also formed by reduction of cathinone during drying and storage (Feyissa and Kelly, 2008, Kalix, 1990, Sporkert et al., 2003).

The objective of this part of study is to extract, identify and determine the khat (Catha edulis) plant.

MATERIALS AND METHODS

MATERIALS

1, 2-Dichlorethan, diethylamine and ninhydrin were purchased from Sigma, USA. Norpseudoephedrine-HCl and norephedrine-HCl were purchased from Nutech, India. Ethanol (95%), methanol and ethyl acetate were purchased from Fisher Scientific, UK. Chloroform and hydrochloric acid were purchased from BDH Laboratory, Poole, England. Glacial acetic acid, sodium hydroxide and ammonium hydroxide were purchased from R & M Marketing, Essex, UK.

COLLECTION AND DRYING OF KHAT LEAVES

The type of khat used in this study is “ Al-Hamdani” khat from Sana’a City, Yemen. Fresh khat leaves and its soft stems were collected in summer and were weighed and washed with distilled water three times. The plants were then left to dry for 3 days in a clean, dry room (20 ± 5°C) and protected from sunlight. After drying, the plant was weighed and packed in a closed foil packet. The foil packets were transported to the laboratory of Pharmaceutical technology discipline, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia, and stored at 5 °C until use.

HERBARIUM STUDY

The imported khat tree samples were classified, photographed and prepared in the herbarium unit in the School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia. The herbarium reference of khat (Herbarium P. P. S. K No. 11019) was kept in the Herbarium Pusat Pengajian Kajihayat, Universiti Sains Malaysia.

EXTRACTION OF DRIED KHAT LEAVES

For each experiment, a new batch of khat was extracted and prepared immediately before use. The procedure for khat extraction was a modified method described by previous workers (Banjaw and Schmidt, 2004, Dimba et al., 2004). Briefly, dried khat leaves (100 g) were chopped into small pieces and added to 100 mL of 95% ethanol, homogenized at 5, 000 rpm for 5 minutes, and filtered with Whatman filter paper (no. 1). This procedure was repeated again with an additional 100 mL of ethanol and was filtered. A total of 200 mL of ethanol extract was concentrated using a rotary evaporator

(EYELA, N-1001S-W, Tokyo, Japan) at 30°C with a rotation speed of 70 rpm until 70% of the ethanol solvent was evaporated. The resulting viscous solution was stirred at 1, 000 rpm with 100 mL of distilled water for 1 hour at ambient temperature. The filtrate was kept frozen at -70°C for 24 hours and dried by lyophilization (Labconco, Kansas City, MO, USA).

ISOLATION OF KHAT ALKALOID

The collected freeze-dried extract powder was partitioned with chloroform and water (1: 1, v/v). The chloroform layer underwent chromatography on a silica gel column and eluted with 150 mL of chloroform, followed by 100 mL of chloroform containing glacial acetic acid (10: 0. 01, v/v). Two parts were collected: part A and part B. Part A underwent further column chromatography and eluted with 100 mL of ethanol containing 5% glacial acetic acid. Upon elution, two portions (1 and 2) were collected. Portion 2 was concentrated and stirred with 100 mL of 0. 1 M HCl, eluted with chloroform, in the presence of diluted 1 M sodium hydroxide (pH 11-12). The solution was precipitated, and a precipitate was collected.

IDENTIFICATION OF KHAT ALKALOID

Thin layer chromatography (TLC) was used to confirm the presence of alkaloid in the khat extract (Dimba et al., 2004). TLC aluminum plates precoated with silica gel (60F-254) were employed as the stationary phase (TLC aluminum sheets 20 Ã- 20, Merck, Darmstadt, Germany). The mobile phase consisted of ethyl acetate: methanol: ammonium hydroxide (85: 10: 5), followed by 0. 5% ninhydrin solution to detect the presence of alkaloid (Szendrei, 1980). The stationary phase was spotted with khat extract, norephedrine, and cathine standard (norpseudoephedrine).

HPLC DETERMINATION OF KHAT ALKALOID

A high-performance liquid chromatography (HPLC) system was comprised of a pump (Gilson, Villiers le Bel, France) equipped with a six-valve sample injection port fitted with a 20-mL sample loop (Rheodyne, PerkinElmer, Cotati, CA, USA), Ultraviolet detector (Model 115, Gilson), and an integrator [D-2500 Chromato-Integrator, (Hitachi, Tokyo, Japan)]. The detector was operated at a detection wavelength of 210 nm. A reversed-phase Luna C18 (5 mm, 150 Ã- 4. 6 mm ID) column (Phenomenex, Torrance, CA, USA), fitted with a refillable guard column (Upchurch Scientific, Oak Harbor, WA, USA) packed with Perisorb RP-18 (30-40 mm, pellicular), was used for chromatographic separation. The mobile phase used for khat extract alkaloid and standards (norpseudoephedrine-HCl and norephedrine-HCl) were consisted of 1, 2-dichlorethan, methanol, acetic acid, diethylamine and water at ratio (v/v) of 800: 200: 10: 5: 7. 5 (Geisshüsler and Brenneisen, 1987). The mobile phase was filtered and degassed by passing through a 0. 45-mm nylon membrane filter (Whatman, Maidstone, UK) under a vacuum before use. The analysis was run at a flow rate of 1. 0 mL/minute and retention times of 13. 45 and 15 minutes for norephedrine and cathine respectively.

The standard stock solutions were prepared by dissolving 20 mg of standards (norpseudoephedrine-HCl or norephedrine-HCl) in 20 mL of methanol to achieve a concentration of 1 mg/mL solution. The working standard solution was then prepared by serial dilution of the stock solution with methanol. The standard calibration curve was constructed at a concentration range of 6. 25-200 mg/mL. The experiments were repeated six times, and the standards were used to determine the within-day and

between-day precision and accuracy of the method. 20 mg of khat extract powder was dissolved in 20 mL of methanol. One mL was further diluted with 9 mL of methanol; 20 Î¼L of sample was injected into the HPLC system.

RESULTS AND DISCUSSION

COLLECTION AND DRYING OF KHAT LEAVES

The yield of dry khat leaves was 220 g per 1000 g of fresh leaves.

KHAT EXTRACTION

For every 100 g of dried leaves, 8. 67 g of khat extract powder was obtained. The ratio of fresh khat leaf to dried khat extract was 100: 1. 91, which was higher than the results published in other studies, 100: 0. 90 (Connor et al., 2002), 100: 0. 90 (Banjaw and Schmidt, 2005), and 100: 0. 58 (Abdulwaheb et al., 2007). This difference could be explained by the fact that only the soft, young stems and leaves were used in the extraction process of this study, whereas other researchers used the entire commercial bundle, including the adult leaves and hard stem fibers (Abdulwaheb et al., 2007, Connor et al., 2000, Connor et al., 2002). The active constituents are concentrated in young leaves, which explain why khat users prefer the young leaves from the tips of the branches (Kalix, 1990).

IDENTIFICATION OF KHAT ALKALOID

TLC revealed that the retention factor (Rf) values for the samples obtained from dried khat extract appeared as two spots at 4 and 4. 5 cm similar to the standards, cathine and norephedrine, respectively. Khat alkaloid; cathine and norephedrine were focus when referred to khat in the present study because TLC analysis of the samples collected from column chromatography

confirmed the presence of cathine and norephedrine. However, no cathinone was found in this test comparing with other TLC result (Szendrei, 1980).

HPLC DETERMINATION OF KHAT ALKALOID

The HPLC results showed that the total percent of cathine and norephedrine in the dried khat extract was about 89%. The composition of alkaloid was represented 81. 3 ± 2. 5% of cathine, and 17. 2 ± 3. 2 of norephedrine.

CONCLUSION

The fresh khat leaves were dried and extracted. The alkaloid content of khat extract was identified and determined. The yield of dried khat leaves was 22% of fresh leaves and khat extract powder was 8. 67% of dry khat leaves. The isolated alkaloid was 89% of the khat extract powder. The composition of alkaloid; cathine, and norephedrine were about 81 and 17% respectively.