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# Stachydrine and Volatile Isothiocyanates from the Unripe Fruit of *Capparis spinosa* L.

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## ABSTRACT

The alkaloid stachydrine of the unripe fruit as well as other parts of *Capparis spinosa* var. *mucronifolia* was extracted by 80% methanol, purified by column chromatography and identified by $^1$H NMR and IR spectroscopic methods. Volatile isothiocyanates were prepared by hydro-distillation of the autolyzed plant material and studied by GC and GC/MS methods.The volatile oil of the unriupe fruit was composed mainly of methyl isothiocyanate (39.2%), isopropyl isothiocyanate(21.4%), and sec-butyl isothiocyanate (6.4%).

**Key words**: Capparis spinosa, Capparidaceae, Stachydrine, Isothiocyanates.

## INTRODUCTION

*Capparis spinosa* var. ***mucronifolia*** (Boiss.) Hedge & Lamond (family: Capparidaceae) grows wild in different parts of Iran (1,2). The floral buds, the unripe fruits, and other fresh aerial parts are stored in vinegar or brined for three months and are used as a pickle in the southern parts of the country. Occurrence of the alkaloid stachydrine in *Capparis spinosa* growing in other parts of the world has been reported (3,4). Since stachydrine containing species are widely used against rheumatism and other diseases(5), therefore it was decided to investigate the occurrence of this active metabolite in *Capparis spinosa* growing wild in Isfahan. Also the flavour profile and identification of some volatile isothiocyanates in the floral bud of *Capparis spinosa* originating from Morocco were investigated(6). This is the first report of the volatile isothiocyanates from the unripe fruit of the same plant growing in Isfahan.

## MATERIALS AND METHODS

**Plant Materials:** Roots, leaves, ripe and unripe fruits of *Capparis spinosa* var. *mucronifolia* (Boiss.) Hedge & Lamond were collected in August 1995 from fruiting plants growing wild in Isfahan (Iran) at an altitude of 1710 m. The plant was identified in the Botany Department, Faculty of Sciences, University of Isfahan, Iran, and a voucher specimen was deposited in the Herbarium of Pharmacognosy Dept, faculty of pharmacy & pharmaceutical sciences, Isfahan university of medical sciences (Iran).

**Extraction and Purification of Stachydrine:** Powdered air-dried samples of the roots, leaves, ripe and unripe fruits (100 gr of each) were separately mixed with 250 ml of 80% methanol and shaked for 24 h. Each methanolic extract was then filtered,and the filterate was shaked gently with petroleum ether (3×25 ml), evaporated, and the residue was dissolved in 15 ml of 2N hydrochloric acid. A small amount of sodium chloride was added and the mixture was left aside at room temperature for 20 h, then filtered. The filterate was made alkaline (pH=10) with 25% ammonia, then shaked with chloroform (3×10 ml), and the aqueous phase was evaporated to dryness. The residue was then dissolved in 15 ml of cooled (0°C) 96% ethanol, filtered, evaporated, and the residue

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was again dissolved in cooled 96% ethanol. The ethanolic solutions of all samples (roots, leaves, ripe and unripe fruits) which had the same TLC profile (silica gel $GF_{254}$, ethanol-water 1:1 as eluent) were fractioned by column chromatography (silica gel for the column chromatography 70-325 mesh) using the same TLC eluent thus obtaining the pure alkaloid.

**Spectroscopic Analyses of Stachydrine**:

The $^1H$ NMR spectra were recorded with a Brucker AC 80 spectrometer, and IR spectra with a Perkin Elmer 1420.

**Autolysis of the Unripe Fruit and Oil Preparation:**

30 gm of the powdered air-dried unripe fruit was mixed with 450 ml of distilled water and left for autolysis at 25°C for 17 hr (7). On the next day, the product was subjected to hydro distillation for 3 h using the apparatus according to the British Pharmacopoeia (8). A yellow volatile oil heavier than water and having pungent smell was collected. The sample oil used for GC and GC/MS analyses was collected in n-pentane.

**GC Analysis:**

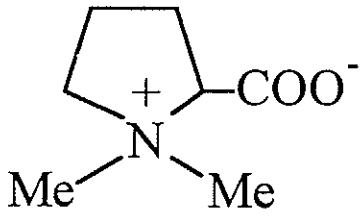
The gas chromatographic determinations were run on a Varian 3400 instrument using a DB-5 capillary column (25m×0.25µm I.D.; film thickness: 0.25 mm). Carrier gas was hel- ium with a flow rate of 1.5 ml/min. The oven temperature was programmed from 60 to 106 °C at 3°C/min, then 106 to 280°C at 6°C/min. Retention indices according to Van den Dool and Kratz were determined using n-alkanes from C6 to C22 as external reference compounds.

**GC-MS Analysis:**

The mass spectra were recorded on a Finnigan MAT Incos-50 instrument. The same DB-5 capillary column was used and the GC conditions were as above. Mass spectrometer conditions were as follows: ionization potential, 70eV; ionization current: 2 A; source temperature, 150°C; resolution, 1000. Identification of the constituents was based on comparison of mass spectra with the literature data (9,10).

## RESULTS AND DISCUSSION

The alkaloid stachydrine had a strong absorption in the IR spectrum at 1625 $cm^{-1}$ due to a COO- group, and suggests a zwitterionic structure of the compound(11). Its $^1H$ NMR spectrum showed signals of two N-methyl




groups at 3.07 and 3.25 ppm as well as signals centered at 2.25 ppm (m,4H),3.56 ppm(m,2H), and 4.05 ppm (dds,1H ) representing the remaining protons of the molecule (11,12). Percentage of the estimated alkaloid in the dried roots, leaves, ripe and unripe fruits were 0.386, 0.410,0.582,and 0.584 respectively. The volatile oil of the unripe fruit of examined *Capparis spinosa* was composed mainly of methyl, isopropyl, and sec-butyl isothiocyanates (see Table 1). The presence of the three mentioned isothiocyanates has been also reported in the floral bud of the same plant growing in Morocco(6). The occurrence of methylglucosinolate, which on hydrolysis produces methyl isothiocyanate, was reported in the same plant growing in Egypt (13), and also in *Capparis baducca*, *Capparis hastata*, *Capparis odoratissima* (14), and *Capparis inermis*(15) growing in other parts of the world. Apparently this glucosinolate is most widely distributed compound in *Capparis* species(16).

**Table 1**:Volatile isothiocyanates of *Capparis spinosa* var. *mucronifolia.*

| Retention indices | $M^+$(%) | Eight main fragment ions(%) | Identity | Relative% |
|---|---|---|---|---|
| 742 | 73(100) | 73(100),72(71),45(21), 44(18),70(11.5),58(9), 36(8),46(6.5). | Methyl isothiocyanate | 39.2 |
| 837 | 101(100) | 101(100),41(83),39(46), 43(40),42(31),86(28), 60(17),59(16). | Isopropyl isothiocyanate | 21.4 |
| 920 | 115(72) | 41(100),115(72),86(68), 56(64),57(63),55(28), 60(28),39(25). | sec-Butyl isothiocyanate | 6.4 |

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