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## Aromatic Compounds: From Plant to Nutraceuticals— An Example of Capsaicin

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Additional information is available at the end of the chapter

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### Abstract

The current study is on extraction of capsaicin from capsicum using chromatography. Nuclear magnetic resonance was used to ascertain carbon structures of the extract, with the yield confirmed by nuclear magnetic resonance to be 98% pure capsaicin. The chemistry, pharmacological action, and side effects of capsaicin are thereafter discussed.

**Keywords:** aromatics, capsaicin, capsaicinoids, chromatography, nutraceuticals, solanaceae

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### 1. Introduction

*Capsicum frutescens* Linn. (Family: Solanaceae) fruits were purchased from Warwick market in Durban, South Africa. The fruits were identified and authenticated by a botanist. A voucher specimen of the plant (with its ripe fruits) has been deposited in the University's Herbarium (JAT/01). The total weight of the dried red chili pepper fruits purchased from the local market was 3 kg. Following cleaning and subsequent pulverization, 2.5 kg of the chili powder was poured into a large conical flask. Thereafter, it was exhaustively extracted sequentially in hexane, dichloromethane, and ethyl acetate. Ethyl acetate extract was shown to contain active compounds (capsaicinoids) in preliminary pilot experiments. Using a rotary evaporator under reduced pressure, the ethyl acetate extract was concentrated to yield 298 g (10% yields) of the crude extract. Following further purification, nuclear magnetic resonance (NMR) analysis of the yield shows it to be 98% pure capsaicin.

## 2. Research methodology

Experimental procedures and protocols used in this study were approved by the Animal Ethics Committee of the University of Durban-Westville, Durban 4000, South Africa (now the University of KwaZulu-Natal, Westville Campus), and conform to the “*Guide to the care and use of animals in research and teaching*” (published by the University of Durban-Westville, Durban 4000, South Africa) [1–7]. Experimental protocols were written so as to lay down the organization of the study. The sequence of this study includes the purchase of *C. frutescens* from a local market, identification of *C. frutescens* by a Botanist, extraction and purification of *C. frutescens* fruit using liquid and paper chromatography.

A portion of the crude ethyl acetate extract was subjected to column chromatography over silica gel with gradient elution using 10% ethyl acetate in hexane to 40% ethyl acetate. On the basis of their thin-layer chromatography (TLC) similarities, a total of 51 eluates (25 ml each) were collected and combined into 15 fractions. These consisted of the non-polar fractions (one to three eluates), which were chlorophylls and its like, which were discarded. Next were the semi-polar/polar fractions (4–15 eluates). The latter were subjected to further column chromatography over silica gel with gradient elution (consisting of 30% ethyl acetate in hexane to 50% ethyl acetate). TLC was carried out on pre-coated aluminum plates by the use of Merck Si gel F254. Following further enhancement, the developed TLC plates were visualized under ultraviolet (UV) light (using wavelength of 254 and 366 nm), by spraying with anisaldehyde/sulfuric acid/alcohol solution, and heating at 110°C for 5 min. The presence of triterpenoids, as previously reported [8–10], was indicated by the appearance of blue to violet-blue coloration. NMR spectra (1D and 2D) were obtained on a Varian 300 (300-MHz) spectrometer, using the residual solvent peaks as internal standards.

In order to ensure that the chromatographic systems were up to standard, phase A of this study was conducted in the Chemistry Department under the supervision of a Professor of Analytical Chemistry. Finally, the identification of capsaicin from *C. frutescens* fruit extract was done by nuclear magnetic resonance in collaboration with the Chemistry Department of the University of KwaZulu-Natal to identify the final extract, that is, capsaicin.

## 3. Discussion

Phytochemical revolution took a positive turn in the 1970s onward, with the development of many laboratories that were involved in the production of bioactive nutraceuticals such as beta-carotene, omega-6, and other vitamins, with positive health-deriving values. Capsaicin is a chili pepper-derived spice. Apart from its spicy and flavorant properties, it has been found to be a digestive aid, a topical painkiller, and a potential cancer-fighting compound [11]. Capsicum is native to America, and the early Spanish explorers brought it to the “New World.” It is cultivated in tropical regions of India, Nigeria, Japan, Southern Europe, Mexico, other African countries, and Sri Lanka.

Capsicum is 5–12 cm long, 2–4 cm wide, and could be globular, cylindrical, oval, or oblong in shape. It has a shriveled shape and could be orange, green, yellow, or red in color, with a prominent and bent pedicle. Internally, the fruits are divided into two halves by a membranous dissepiment to which the seeds are attached. Capsicum has a characteristic odor and an intense pungent taste. Although *Capsicum* can withstand tropical heat, it requires 3 months rainfall and thrives best in wet climate. The fruit yield is directly proportional to the manure of the cultivated farm.

The fruits are picked as they become fully ripe. The unripe fruits fade upon drying. The fruits are dried in sun and graded by color. The quality of the fruit is in part determined by its color, and they are occasionally oiled to give glossiness to their pericarps.

The pungent compounds of *C. frutescens* are capsaicin (69%), dihydrocapsaicin (22%), nordihydrocapsaicin (7%), homocapsaicin (1%), and homodihydrocapsaicin (1%). Capsaicin consists of an aromatic portion, which is derived from phenylalanine through frulic acid and vanillin—an aldehyde is a substrate for transamination to give vanillylamine. The acid portion of the amide structure in capsaicin is of polyketide origin, with a branched-chain fatty acyl-CoA, which is produced by a chain extension of 2-methylacetyl-CoA, with a starter unit, which is valine-derived.

In this study, vanilloid alkaloids otherwise known as capsaicinoids were extracted by sequentially in hexane, dichloromethane, and ethyl acetate. The extracts were later subjected to liquid, paper, gas chromatography, and high-performance liquid chromatography (HPLC).

The yield of 298 g (10% yields) of the 2.5-kg fruit is consistent with standard industrial yield. Following further purification, NMR analysis of the yield shows 98% capsaicin.

In 1912, Wilbur Scottville developed a dilution taste method, called “Scottville Organoleptic Test (SOT),” to measure the heat level of a chili pepper. This test consists of blended pure ground chilies with sugar water solution. In addition, there is a panel of testers who will sip the concoctions. Scottville increasingly diluted the concentrations until they reached the point at which the liquid no longer burned the mouth. Based on how much the chili needed to be diluted before one could taste no heat or burn the mouth, a number was then assigned to each chili to show its strength.

Measurement of the pungency of chili is done in multiples of 100 units. While the bell pepper measures zero Scottville units, the incendiary Habanero measures 300,000 Scottville units. For comparison, one part of chili “heat” per 1,000,000 drops of water rates as only 1.5 Scottville units. In another respect, while the substance that makes chili so hot and therefore so enjoyable is capsaicin, a pure capsaicin rates over 150,000,000 Scottville units. Red Savina Habanero is much hotter than the normal Habanero with “Guinness Book of Records” of 577,000 Scottville units while the Trinidad scorpion Moruga tests over two million Scottville units. The validity and accuracy of the Scoville Organoleptic Test (SOT) have been widely criticized. The Gillet method adopted by the American Spice Trade Association and the International Organization for Standardization is a modified version of the SOT. Although very costly, the high performance liquid chromatography is the most objective analysis method for obtaining the purest

form of capsaicin. Capsaicin (N-vanillyl-8-methyl-6- (E)-nonenamide) is the most pungent of all the groups of compounds called “capsaicinoids” that can be isolated from chili peppers. It is sparingly soluble in water but very soluble in fats, oils, and alcohol.

Chromatographic separation can be achieved using three contrasting modes. They are column chromatography, thin-layer chromatography, and paper chromatography. In column chromatography, the stationary phase is attached to a suitable matrix (an inert, insoluble support) packed into a glass or a metal column. The mobile phase is passed through the column either by gravity feed (as in this study) or by the use of a pumping system, or applied gas pressure [9, 10]. This is the most common form of chromatographic modes. In thin-layer chromatography, the stationary matrix is coated thinly on to a glass, plastic, or metal foil plate. The mobile liquid phase across the thin-layer plate held either horizontally or vertically is by capillary action. Its advantage is that a large number of samples can be studied simultaneously. In paper chromatography, the stationary liquid phase is supported by the use of cellulose fibers of a paper sheet. Thin-layer and paper chromatography have several similarities. In both modes, the mobile phase passes along the paper sheet either by gravity feed or by capillary action and are the older forms of chromatography, with few current serious biochemical applications.

## 4. Conclusion

The result shows an extraction yield of 12%, which compares favorably with standard extraction process. An alternative source of the nutraceutical is shown in this study. NMR remains a gold standard in ascertaining the carbon-atom structure and high purity yield despite the use of high performance liquid chromatography (HPLC).

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