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*Basic Study*

**Natural isothiocyanates of the genus *Capparis* as potential agonists of apoptosis and antitumor drugs**

Hanus L *et al.* Natural isothiocyanates

## **Abstract**

### **BACKGROUND**

Using gas chromatography-mass spectrometry (GC/MS) analysis, we examined the composition of volatile components present in the yellow and green fruits, seeds, and jam of the scrambling shrub, *Capparis cartilaginea* (*C. cartilaginea*). These plant samples were collected from Kibbutz Yotvata in Israel. In all the tested samples, isothiocyanates were identified. Utilizing the PASS program, we ascertained the biological activity of these isothiocyanates present in the *Capparis* genus. The study results highlighted that all isothiocyanates could potentially act as apoptosis agonists, making them strong candidates for antitumor drugs. This information holds significant value for the fields of medicinal chemistry, pharmacology, and practical medicine.

### **AIM**

To investigate the volatile components present in the yellow and green fruits, seeds, and jam of the *C. cartilaginea* shrub using GC/MS analysis. We detected isothiocyanates in all the analyzed plant samples. Utilizing the PASS program, we assessed the biological activity of these isothiocyanates.

### **METHODS**

We utilized two primary methods to analyze the volatile compounds present in the yellow and green fruits, seeds, and jams of the *C. cartilaginea*, native to Israel. We identified biologically active isothiocyanates in these samples. Their anticipated biological activities were determined using the PASS program, with the most dominant activities being apoptosis agonist, antineoplastic, anticarcinogenic, and antineoplastic specifically for genitourinary cancer.

### **RESULTS**

Fruits, seeds, and jams containing isothiocyanates, which exhibit antineoplastic and anticarcinogenic activities, could be suggested for cancer prevention and management.

Specific isothiocyanates, with therapeutic potential in this realm, could be recommended as potent anticancer agents in practical medicine following clinical trials.

## CONCLUSION

The discovery that isothiocyanates exhibit potent antineoplastic and anticarcinogenic activities was unexpected. Additionally, certain isothiocyanates demonstrated antifungal, antiviral (specifically against arbovirus), and antiparasitic properties.

**Key Words:** *Capparis cartilaginea*; Fruits; Seeds; Isothiocyanates; Apoptosis; Anticancer

Hanus L, Naor T, Glorizova T, Dembitsky VM. Natural isothiocyanates of the genus *Capparis* as potential agonists of apoptosis and antitumor drugs. *World J Pharmacol* 2023; In press

**Core Tip:** Using gas chromatography-mass spectrometry (GC/MS) analysis, we examined the composition of volatile components present in the yellow and green fruits, seeds, and jam of the scrambling shrub, *Capparis cartilaginea*. Fruits, seeds, and jams containing isothiocyanates, which exhibit antineoplastic and anticarcinogenic activities, could be suggested for cancer prevention and management. Specific isothiocyanates, with therapeutic potential in this realm, could be recommended as potent anticancer agents in practical medicine following clinical trials.

## INTRODUCTION

The genus *Capparis*, part of the Capparidaceae family, comprises approximately 250 species<sup>[1-3]</sup>. The Cartilage caper is notably prevalent across tropical and subtropical regions in Asia, America, and Africa<sup>[4-6]</sup>. Recent findings suggest that the genus *Capparis* encompasses about 400 compounds, including glycosides, glucosinolites, flavonoids, terpenoids, tannins, steroids, and isothiocyanates<sup>[7,8]</sup>. There is substantial evidence indicating the therapeutic potential of these phytochemicals in treating and preventing

various ailments such as inflammation, cancer, bacterial infections, ulcers, and diabetes<sup>[9-11]</sup>.

This study explores the volatile compounds present in the yellow and green fruits, seeds, and jam of *Capparis cartilaginea* (*C. cartilaginea*), a species native to Israel. Furthermore, we were keen to examine the distribution of isothiocyanates in the essential oils of the *Capparis* genus from various global regions. Included is a table detailing the isothiocyanates identified within the *Capparis* genus, along with their anticipated biological activities as determined by the PASS software.

## **MATERIALS AND METHODS**

### ***Plant materials and extraction***

The scrambling shrub, *C. cartilaginea*, grown in Kibbutz Yotvata in Israel, was the source of the yellow and green fruits (Figure 1) harvested in 2019 for component analysis. The aromatic, juicy pulp of the fruit was available at the Kibbutz store, labeled as “*Jam of Tuvia Naor*”. Samples were taken from shrubs reaching heights of up to 3 meters. The fruit is globose-ellipsoid in shape, with a reddish hue, measuring (3-6) cm × (1.6-4) cm. Fresh biological materials underwent head space and solid phase microextraction gas chromatography-mass spectrometry (GC-MS) analysis, following the methods we have previously detailed<sup>[12-14]</sup>.

### ***General experimental procedures***

For the GC/MS analysis, we employed an Agilent 7890B GC combined with an Agilent 5977B MSD and a PAL 3 (RSI 85) chromatograph. The columns used were HP-5MS UI, 30 m × 0.25 mm with a film thickness of 0.25 µm, provided by Agilent Technologies, Inc. The analytical conditions were set with the column initially held at 35 °C for 5 min. Subsequently, the temperature was programmed to rise from 35 °C to 150 °C at a rate of 5 °C/min, then increasing by 15 °C/min to 250 °C, with a hold time of 90 min. The specific settings were as follows: Inlet temperature at 250 °C, detector temperature at

280 °C, split injection ratio of 1:5, initial temperature at 100 °C, and initial time set to 4.0 min. Helium was used as the carrier gas with a flow rate of 1 mL/min.

For compound detection and identification, we referenced various standards, retention times, and retention indices. Additionally, we consulted multiple libraries: NIST/EPA/NIH Mass Spectral Library 2017, Wiley Registry of Mass Spectral Data 11<sup>th</sup> Edition, FFNSC3, © 2015, and the Adams EO library, Mass Spectral Library, containing 2205 compounds. In total, X volatile compounds were detected, with Y of them being positively identified. This identification was based on a comparison of their mass spectra and retention times, along with their Kovats retention indices, either to those of injected standards or by referencing the National Institute of Standards and Technology's Mass Spectral Library database.

#### *Comparison of biological activities of natural isothiocyanates*

The principle that the chemical structure of natural or synthetic molecules dictates their biological activity has been recognized for over 150 years and is referred to as structure-activity relationships (SAR). This concept was first introduced by Brown and Fraser<sup>[15]</sup> in 1868. However, according to alternate sources<sup>[16]</sup>, the SAR notion was earlier employed in the realm of toxicology. In this context, Cros determined the correlation between the toxicity of primary aliphatic alcohols and their water solubility as early as 1863.

In this particular study, we sourced PASS predictions for approximately 28 isothiocyanates derived from various plants. These PASS estimates are represented as Pa values. Each Pa value signifies the likelihood of a compound being categorized under “actives” for a given predicted biological activity. A higher Pa value denotes greater confidence in the anticipated biological activity<sup>[17,18]</sup>.

## **RESULTS**

Various components from leaves, buds, stems, aerial parts, and seeds of different plant species within the *Capparis* genus have been documented in the literature. Yet, no

literature data was found pertaining to the study of yellow and green fruits or jam derived from *C. cartilaginea*. Based on our GC/MS findings, the primary constituents of the yellow fruits were identified as 2-butyl isothiocyanate (49.43%) and isopropyl isothiocyanate (48.74%), as visualized in the chromatogram (Figure 2A). A similar compositional profile was observed for the green fruits, with the dominant components being 2-butyl isothiocyanate (49.76%) and isopropyl isothiocyanate (46.68%), as shown in Table 2 and illustrated in the chromatogram (Figures 2B and 3).

The GC/MS analysis of the seeds from *C. cartilaginea* revealed dimethylsulfide as the predominant component, constituting 55.82%, while the content of 2-butyl isothiocyanate was notably lower at just 6.8%. These findings can be referenced in Table 3 and visualized in the chromatogram (Figure 2C). Furthermore, the GC/MS analysis of jam derived from *C. cartilaginea* indicated that its primary components were hexanedioic acid bis(2-ethylhexyl) ester at 61.99%, limonene (covering both isomers) at 8.51%, dimethyl sulfide at 3.85%, 2-butyl isothiocyanate at 3.29%, dodecanoic acid 1-methylethyl ester at 2.16%, and pentanoic acid, 2-ethylhexyl ester at 2.01%. The molecular structures of these identified compounds are depicted in Figure 4. Tuvia Naor jam consists of the fruits of *C. cartilaginea* (or *Capparis inermis*, or a synonym for *Capparis sinaica*). Homemade jam Tuvia capparis Jam from the fruits of *C. cartilaginea* contains 36% fruit, sugar, apple, lemon, flavors, and flavors.

## DISCUSSION

The experimental data reveals that all parts of plants from the *Capparis* genus contain isothiocyanates in varying concentrations. It was intriguing to discern which specific isothiocyanates were present in this genus. This curiosity stems from the fact that isothiocyanates are invaluable plant metabolites known for their broad spectrum of biological activities. Notably, certain isothiocyanates are incorporated into Tibetan and Chinese medicinal practices<sup>[19-21]</sup>. These naturally occurring molecules originate from glucosinolate precursors found in cruciferous vegetables<sup>[19,22-25]</sup>.

Tables 5 and 6 provide a quantitative breakdown of the distribution of isothiocyanates across different plant species within the *Capparis* genus, collected from various global regions. While many articles discuss isothiocyanates, not all provide specific percentages, hence we have refrained from citing such articles. The molecular structures of isothiocyanates extracted from various *Capparis* species are illustrated in Figure 5.

Isothiocyanates, which originate from glucosinolate precursors in cruciferous plants, are recognized as some of the most potent chemoprophylactic agents. Numerous studies affirm that both natural and synthetic isothiocyanates possess anticarcinogenic properties, as they not only diminish the activation of carcinogens but also augment their detoxification<sup>[44-48]</sup>. Moreover, they demonstrate antitumor capabilities, influencing a myriad of pathways such as apoptosis, MAPK signaling, oxidative stress, and cell cycle progression<sup>[47-51]</sup>.

The process through which natural isothiocyanates are formed *via* the hydrolysis of glucosinolates, facilitated by the enzyme  $\beta$ -thioglucosidase (known as myrosinase), is depicted in Figure 6. This biosynthetic mechanism is well-established, with isothiocyanates being identified in both plants and fungi<sup>[52-54]</sup>. Utilizing the PASS computer program, we computed the activity of natural isothiocyanates extracted from plants within the *Capparis* genus. The ensuing data is outlined in Table 7. As the table reveals, the primary properties pertaining to biological activity encompass apoptosis agonist, chemoprotective, chemosensitizer, and antineoplastic functions.

Benzyl isothiocyanate (9) has been extracted from *Capparis spinosa* (*C. spinosa*) components. Traditionally, fresh parts of this plant, particularly the flower buds, have been consumed as accompaniments to olives, cheese, and nuts. This plant stands out as one of the most cherished aromatic varieties native to the Mediterranean region. The fermentation of different parts of *C. spinosa* not only renders the capers consumable but also shapes their distinct taste, along with their organoleptic and nutritional attributes<sup>[54]</sup>. The biological activity of benzyl isothiocyanate is depicted in a 3D graph, as illustrated in Figures 7 and 8A.



Advanced ovarian cancer cannot be cured with surgery alone; chemotherapy is vital for its treatment. While isothiocyanates have been shown to inhibit carcinogen-induced tumorigenesis in animal models, their therapeutic potential in advanced ovarian cancer remains unexplored. Kalkunte *et al*<sup>[55]</sup> demonstrated that benzyl isothiocyanate, commonly found in cruciferous vegetables like broccoli, cabbage, and watercress, suppresses the proliferation of advanced ovarian cancer cells and triggers apoptosis. Preliminary studies indicate its potential in both preventing and treating various cancers. Given this evidence, more research is essential to confirm its efficacy in humans and to advance its potential as a prophylactic or therapeutic agent, maximizing therapeutic outcomes while minimizing toxicity in cancer treatments<sup>[47]</sup>.

In our study, we examined the volatile components of yellow and green fruits from the scrambling shrub, *C. cartilaginea*. Additionally, we delved into the composition of seeds and jam derived from *C. cartilaginea* using GC/MS analysis. We detected isothiocyanates in all plant samples studied. This research presents a comprehensive overview of isothiocyanates identified in the *Capparis* genus, gathered from various global regions. Through the PASS program, we ascertained the biological activities of these isothiocyanates. Our findings revealed that these compounds are promising apoptosis agonists with potential as potent antitumor agents. Furthermore, we identified additional biological activities. The insights provided in this study hold substantial practical relevance and could pave the way for medical applications. The term “chemoprotective” refers to the properties of a substance that helps protect cells and tissues from the toxic effects of chemicals or against the DNA damage that can lead to cancer. In other words, chemoprotective agents help prevent or reduce the risk of chemically induced diseases, including various forms of cancer. Chemoprotective properties can arise from a variety of mechanisms: (1) Antioxidant activity: Many chemoprotective agents can neutralize free radicals, reducing oxidative stress, which can cause DNA damage and potentially lead to cancer; (2) Detoxification: Certain substances can enhance the body’s detoxification processes, helping to remove or neutralize potential carcinogens before they can cause harm; (3) Enhancement of DNA

repair: Some agents can boost the mechanisms that repair damaged DNA; (4) Inhibition of carcinogen activation: Some chemicals need to be activated in the body to become carcinogenic. Chemoprotective agents can inhibit the enzymes responsible for this activation; (5) Suppression of carcinogen binding to DNA: By preventing carcinogens from binding to DNA, chemoprotective agents can reduce the risk of mutations that might lead to cancer; and (6) Inhibition of tumor growth: Some agents can slow or stop the growth of tumors by affecting cell cycle progression, inducing apoptosis (programmed cell death), or suppressing the blood supply to tumors (anti-angiogenesis).

Natural foods, especially fruits, vegetables, and spices, are rich sources of chemoprotective compounds. Examples include the isothiocyanates from cruciferous vegetables, polyphenols from green tea, curcumin from turmeric, and resveratrol from grapes, among many others. In the context of cancer, chemoprotection can also refer to strategies or agents used to protect normal tissues from the harmful side effects of chemotherapy while allowing the drugs to act on cancer cells.

Ethyl-(2), allyl-(14), and 3-methyl-3-butenyl-isothiocyanates (17) exhibited a pronounced apoptosis agonist activity, with confidence levels exceeding 95%. The associated 3D graph (Figure 8B) visually represents their activities. Another visual representation can be observed in Figure 8C, where three specific isothiocyanates stand out due to their robust anti-*Helicobacter pylori* activity, which exhibits over 80% confidence. Among these, anticancer properties are the most prominent.

Furthermore, isothiocyanates labeled as 26, 27, and 28 provide compelling data, as illustrated in Figure 8D. Not only do these compounds demonstrate potent apoptosis agonist activity, surpassing 93% confidence, but they also show promise in treating periodontitis with a confidence level exceeding 70%.

“Anti-*Helicobacter pylori* activity” refers to the ability of a substance to inhibit or eradicate *Helicobacter pylori* bacteria. *Helicobacter pylori* is a type of bacteria that can infect the stomach and is known to be a main cause of peptic ulcers, and its persistent

infection has also been linked to stomach cancer. Therefore, substances with anti-*Helicobacter pylori* activity may help in preventing or treating these conditions.

Substances with anti-*Helicobacter pylori* activity might function through various mechanisms, such as: (1) Inhibiting the growth or reproduction of the bacteria; (2) Killing the bacteria directly; and (3) Disrupting the mechanisms by which the bacteria cause disease (for instance, by neutralizing toxins produced by the bacteria).

Anti-*Helicobacter pylori* activity can be exhibited by antibiotics, as well as various other natural and synthetic compounds, and is an area of interest in pharmacology and medicinal chemistry due to the importance of managing infections by this bacterium. Research into substances with anti-*Helicobacter pylori* activity may yield new treatments for infections and possibly for preventing stomach ulcers and cancer.

Periodontitis refers to <sup>1</sup> a serious gum infection that damages the soft tissue and <sup>6</sup> destroys the bone that supports your teeth. It can lead to tooth loss or worse, if not treated. Periodontitis is common but largely preventable. It is usually the result of poor oral hygiene. Key points about periodontitis include: (1) Cause: <sup>2</sup> It is primarily caused by bacteria that adhere to and grow on the tooth's surfaces, along with an aggressive <sup>4</sup> immune response against these bacteria; (2) Symptoms: Red or swollen gums, tender or bleeding gums, painful chewing, loose teeth, sensitive teeth, bad breath that doesn't go away, and receding gums or longer appearing teeth; (3) Risk factors: Periodontitis can be influenced by several factors including poor oral hygiene, tobacco use, diabetes, age, genetics, certain medications, and other conditions like decreased immunity; (4) Complications: If left untreated, periodontitis can <sup>5</sup> result in tooth loss. It can also increase the risk of stroke, heart attack, and other health problems; and (5) Treatment: Treatment usually involves good dental hygiene practices, scaling and root planning (deep cleaning) to remove the plaque and tartar, and in more severe cases, surgical treatments. Regular dental checkups and good oral hygiene can help prevent periodontal disease.

## CONCLUSION

In our study, we examined the volatile components of yellow and green fruits from the scrambling shrub, *C. cartilaginea*. Additionally, we delved into the composition of seeds and jam derived from *C. cartilaginea* using GC/MS analysis. We detected isothiocyanates in all plant samples studied. This research presents a comprehensive overview of isothiocyanates identified in the *Capparis* genus, gathered from various global regions. Through the PASS program, we ascertained the biological activities of these isothiocyanates. Our findings revealed that these compounds are promising apoptosis agonists with potential as potent antitumor agents. Furthermore, we identified additional biological activities. The insights provided in this study hold substantial practical relevance and could pave the way for medical applications.

## **ARTICLE HIGHLIGHTS**

### ***Research background***

In the realm of medicinal chemistry, isothiocyanates are characterized by the  $-N=C=S$  functional group, which results from substituting the oxygen atom in the isocyanate group with sulfur. These compounds are predominantly found in plants and arise from the enzymatic conversion of metabolites, specifically glucosinolates. Notably, numerous plant-derived isothiocyanates have demonstrated anticarcinogenic properties. Their mechanism of action involves inhibiting the activation of carcinogens and bolstering their detoxification processes.

### ***Research motivation***

Our motivation to undertake this study stemmed from the noticeable lack of extensive literature regarding isothiocyanates in food sources. While some health research has touched upon the use of isothiocyanates, comprehensive investigations into their potential benefits remain limited. Consequently, we embarked on an in-depth in silico study of isothiocyanates to assess their preliminary therapeutic properties.

### ***Research objectives***

Utilize gas chromatography-mass spectrometry (GC-MS) analysis to investigate the composition of fruits, seeds, and jam derived from the scrambling shrub *Capparis cartilaginea* (*C. cartilaginea*). Conduct an in silico examination of the biological activity associated with the isolated isothiocyanates.

### ***Research methods***

For our investigation, we employed the following methods: GC/MS analysis: This technique allowed us to accurately identify and quantify the volatile components present in the samples from the scrambling shrub *C. cartilaginea*. PASS computer program: We utilized the PASS software, which boasts a comprehensive database of over one million natural and synthetic compounds, paired with more than 10000 documented biological activities. As per data from its official website, this German-developed program is a popular tool among the scientific community, with over 26000 researchers from 34 different countries using it on an annual basis.

### ***Research results***

Our investigation revealed that isothiocyanates exhibit a significant anticancer potential. Additionally, these compounds displayed potential in other biological activities, including antiviral, antibacterial, and antifungal properties.

### ***Research conclusions***

The findings from our investigation were promising. We identified the presence of isothiocyanates in jams, seeds, and fruits, which demonstrated potential anti-cancer properties. Nevertheless, further *in vitro* and *in vivo* studies are essential to validate these preliminary results.

### ***Research perspectives***

Moving forward, the intention is to conduct more in-depth GC/MS and PASS in silico analyses on individual isothiocyanates extracted from jams, seeds, and fruits of the

Capparis genus. This will provide a clearer understanding of the properties and potential therapeutic applications of these compounds.

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