Original Research

Sourcing the Antifeedant Properties of Pineapple Weed (*Matricaria discoidea*)

L'approvisionnement des propriétés antialimentaires de la matricaire odorante (*Matricaria discoidea*)

Josiah A. W. Smith[1], Sharon Barden[1], Paul M Mayer[1*]

1. University of Ottawa, Ottawa, ON, Canada
*Corresponding author. Email: pmmayer@uottawa.ca

Abstract | Résumé

This study investigated the chemical composition of *Matricaria discoidea*, commonly known as pineapple weed, for the purpose of analyzing potential medicinal applications. A gas chromatograph mass spectrometer was utilized to identify the compounds in acetonitrile, CO_2, and hydrosol extracts of the plant. It was found that the acetonitrile and CO_2 extracts contained various expected molecules, including terpenes and terpenoids, commonly present in plant concentrates. However, the most significant finding was the presence of the two isomers of tonghaosu (1,6-dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiynylidene)). The hydrosol was found to have a composition of solely tonghaosu, promoting further applications due to its isolation. The presence of this molecule reinforces pineapple weed's insect-repelling activity. Consequently, the sizeable quantities of tonghaosu in pineapple weed hydrosol extract establish a promising source of the bioactive compound, supporting a potential for use in natural insect repellent and additional medicinal applications.

Cette étude a examiné la composition chimique de *Matricaria discoidea*, communément appelée la matricaire odorante, dans le but d'analyser les applications médicinales potentielles. Un chromatographe en phase gazeuse couplé à un spectromètre de masse a été utilisé pour identifier les composés dans les extraits d'acétonitrile, de CO_2 et d'hydrolat de la plante. Il a été constaté que les extraits d'acétonitrile et de CO_2 contenaient diverses molécules attendues, notamment des terpènes et terpénoïdes, couramment présents dans les concentrés végétaux. Cependant, la découverte la plus significative a été la présence des deux isomères de tonghaosu (2-(2,4-hexadiynylidène)-1,6-dioxaspiro[4.4]non-3-ène). Il a été constaté que l'hydrosol avait une composition uniquement de tonghaosu, favorisant de nouvelles applications en raison de son isolement. La présence de cette molécule renforce les propriétés anti-insectes de la matricaire odorante. Par conséquent, les quantités importantes de tonghaosu dans l'extrait d'hydrosol de la matricaire odorante établissent une source prometteuse de ce composé bioactif, soutenant un potentiel d'utilisation dans les répulsifs naturels et des applications médicinales supplémentaires.

Keywords: Matricaria discoidea; pineapple weed; tonghaosu; antifeedant compounds; GC-MS; hydrosol extraction; botanical insecticides; phytochemical analysis; natural insect repellents; supercritical CO_2 extraction

Introduction

Matricaria discoidea, commonly referred to as pineapple weed, is a small plant belonging to the Asteraceae family (1). Originating in northwest North America and northeastern Asia, this weed has spread widely across America and Canada, growing wild in fields as well as throughout urban settings (Figure 1), able to reach 12 inches in height (1). This common plant is widely recognized for its fragrant and edible properties (1,2). When crushed, it emits a tropical and fruity aroma with an undertone of chamomile, a closely related organism (1,2). Pineapple weed can be eaten cooked or raw, with the leaves giving a fresh and bitter taste, whereas the flowers are recognized for their herbaceous sweetness (3). Shared with chamomile, pineapple weed also demonstrates medicinal behaviour, helping with digestion and stress in addition to its physical analgesic application to treat sores, bites, and irritated skin (1,2,4). In terms of consumption forms, it is commonly steeped into a tea, used as a garnish, or possibly concentrated into a syrup for flavouring (2). Beyond its ingestion benefits, pineapple weed's aerial components can be burned, rubbed on skin, or sprayed as an extract to repel mosquitoes (2). A distinctive aroma, taste, and biological activity like the one demonstrated by pineapple weed suggest a rich chemical composition, encouraging further analysis to connect structure to its properties.

The chemical composition of *Matricaria discoidea* has been revealed to be similar to chamomile (1,2). A previous study on the essential oil of pineapple weed has identified several terpenes as primary constituents, including myrcene, [E]-beta-farnesene, and germacrene D, with the three compounds making up a

Figure 1. Pineapple weed growing next to a sidewalk

approximately sixty percent of the oil (1). These molecules are responsible for the distinct aroma and additional medicinal properties found throughout terpenes and terpenoids. However, the essential oil of a plant mainly focuses on the composition of volatile and water-soluble components; thus, the chemical background for pineapple weeds' various properties remains an area open to further investigation. In particular, we are looking into the origin of the plant's antifeedant properties, 1,6-dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiynylidene). Also known as tonghaosu, this compound is recognized as an unsaturated spiroketal enol ether with two isomers (Figure 2) (5).

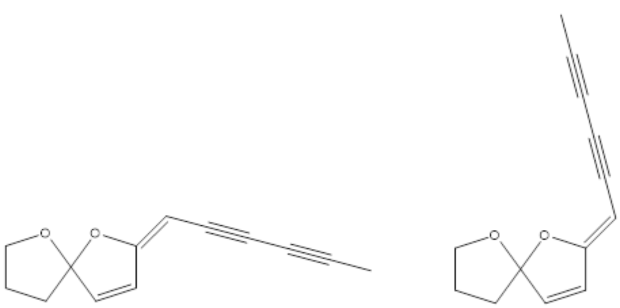

Figure 2. Chemical structure of E-Tonghaosu (left) and Z-Tonghaosu (right)

The history of these molecules originates with a very common vegetable in southern China, the tonghao plant, recognized for its specific odor and immunity to insects (5). Tonghaosu has been discovered in various plants such as Chrysanthemum coronarium L., Dendranthema indicum, and others from the Asteraceae family, including the Chrysanthemum and Matricaria genuses (5). In addition, the cis- or Z-isomer of tonghaosu has been found in German chamomile, a plant with a very close relation to pineapple weed, being a part of the same matricaria genus (2). With such a wide range of plants containing this very particular compound, it suggests that the antifeedant properties exhibited by tonghaosu might have had a survival impact on the evolution and diversification of these plants. It has been found that the antifeedant properties of the Z-isomer of tonghaosu often surpass those of the E-isomer when tested on large white butterflies; however, specific quantified data is not available (6). While no specified data relating to the particular mechanism of tonghaosu is accessible, antifeedant species often work by interacting with sensory cell receptors in insects as a deterrent instead of toxicity (7).

Given the apparent bioactivity of *M. discoidea*'s chemical composition and the significant potential impacts of specific compounds such as tonghaosu present in closely related plant species (8), an in-depth study of pineapple weeds' chemical profile is beneficial. Thus, the purpose of this study is to identify and distinguish chemical constituents of *Matricaria discoidea* using gas chromatography-mass spectrometry, solution extracts, hydrosols, solid phase extraction, and supercritical-CO_2 extractions. During this procedure and study, an emphasis was placed on the presence and abundance of tonghaosu isomers, aiming to provide more information on its chemical availability for traditional applications of the plant and further natural product development.

Experimental Procedures

Acetonitrile Extracts

Pineapple weed was harvested from a suburban sidewalk in Ottawa, Ontario, Canada. Some of the material was then dehydrated and ground into a powder using an electronic grinder. Using an electronic scale, 1 g of the powdered material was weighed and placed into a 10 mL vial. The remaining fresh material was then separated into its head (flower) and leaf (not including stem) components, placing 1 g of each into two separate 10 mL vials. 9 mL of acetonitrile was then pipetted into each vial, shaken, and allowed to sit at room temperature for 4 hours with agitation every 30 minutes.

Hydrosol Extract

250 g of fresh pineapple weed aerial segments were harvested from a relatively low-traffic area and placed in a 250 mL beaker that is part of the microwave distillation apparatus. A distilled water ice cone was screwed onto the inner surface of the apparatus's lid, and a funnel was used to direct the ice water into the beaker. The material was subjected to four 7-minute heating cycles, replacing the ice cone each time. The collected solution was

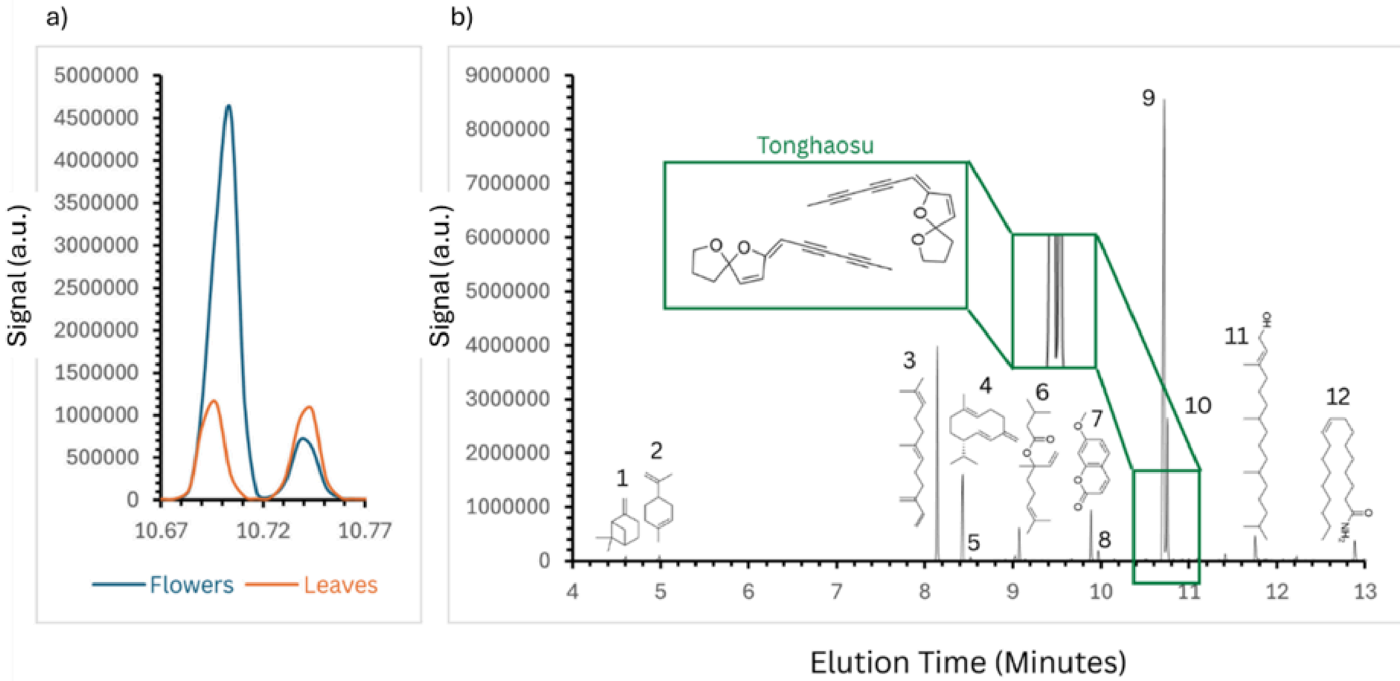

Figure 3. GC-MS chromatogram for *Matricaria discoidea* acetonitrile extracts a) zoom-in on the Tonghaosu region and comparison of separate leaves and flower extracts and b) total extract.

Table 1. Compounds Found in Pineapple Weed Acetonitrile Extract.

Peak Number	Name (Common)	Area %	Elution Time (min)	Retention Index
1	b-Pinene	0.68	4.605	
2	Limonene	0.79	4.988	10.34
3	Beta-Farnesene	16.22	8.142	14.58
4	Germacrene-D*	6.72	8.431	15.02
5	Elixene*	Traces	8.520	15.17
6	Linalyl iso-valerate*	2.47	9.070	16.07
7	Herniarin*	4.28	9.890	17.49
8	Unidentified	0.80	9.971	17.64
9	**Tonghaosu Isomer 1 (Z)**	**50.85**	**10.717**	**19.04**
10	**Tonghaosu Isomer 2 (E)**	**12.73**	**10.751**	**19.11**
11	Phytol	2.41	11.753	21.16
12	Oleamide*	2.03	12.883	23.71

*Unable to definitively confirm identity; however, high percentage quality reports suggest promising specifications.

run through solid phase extraction (Chromosep C18 500mg/6ml, PK50 SPE Column) Compounds were eluted with a 1:1 solution of acetonitrile and methanol prior to GC-MS analysis.

Supercritical-CO_2 Extraction

15 g of fresh pineapple weed was harvested from a low-traffic area, and the aerial segments (flowers, leaves, stems, not roots) were dried. The material was then run through a supercritical CO_2 extractor with an amber vial attached to the output. 1 mL of ethyl acetate was then pipetted into a 2 mL vial, and using a new pipette tip, the CO_2 extract was scraped off the amber vial and mixed into the ethyl acetate. The vial was then agitated until the material was fully dissolved. The procedure was repeated using 10 g of fresh pineapple weed flowers.

GC-MS Analysis

Solutions were filtered, and 1 μL injected onto the GC-MS. The GC-MS conditions were: temperature ramp 40 - 300 °C at a rate of 20 °C per minute; inlet temperature: 200 °C; inlet pressure: 13.96 psi; DB-5 column. Compounds were identified by comparing their mass spectra to those in the NIST mass spectral database. The retention indices were calculated using a C8-C40 standard.The hydrosol was quantified using an internal standard (4-ethylguaiacol).

Results and Discussion

As shown in Figure 3, the pineapple weed extract contains various chemicals (Table 1), including terpenes and terpenoids. However, the most abundant molecules are the two isomers of tonghaosu. The separated flower and leaf extracts demonstrate a large difference in isomer quantities with the flowers having a 6:1 peak area ratio, while the leaves have an even ratio between the two isomers. Overall, tonghaosu represents about 60 % of the derived extract.

The use of gas chromatography-mass spectrometry allowed for a comprehensive analysis of the volatile compounds in *Matricaria discoidea* (pineapple weed). Notably, tonghaosu, a molecule found in related species, was identified as a major component of the plant's aerial segments. As observed, this compound's characteristic double peak on the chromatogram indicates the existence of two isomers, identified to be Z and E structures. To identify each peak, their retention index was calculated by comparison to the C8-C40 standard mixture, and then to the NIST Chemistry Webbook. It was found that the Z-isomer elutes first (9). The Z-isomer comprises approximately 51% of the volatile extract, and the E-isomer represents 13%. However, when analyzing the

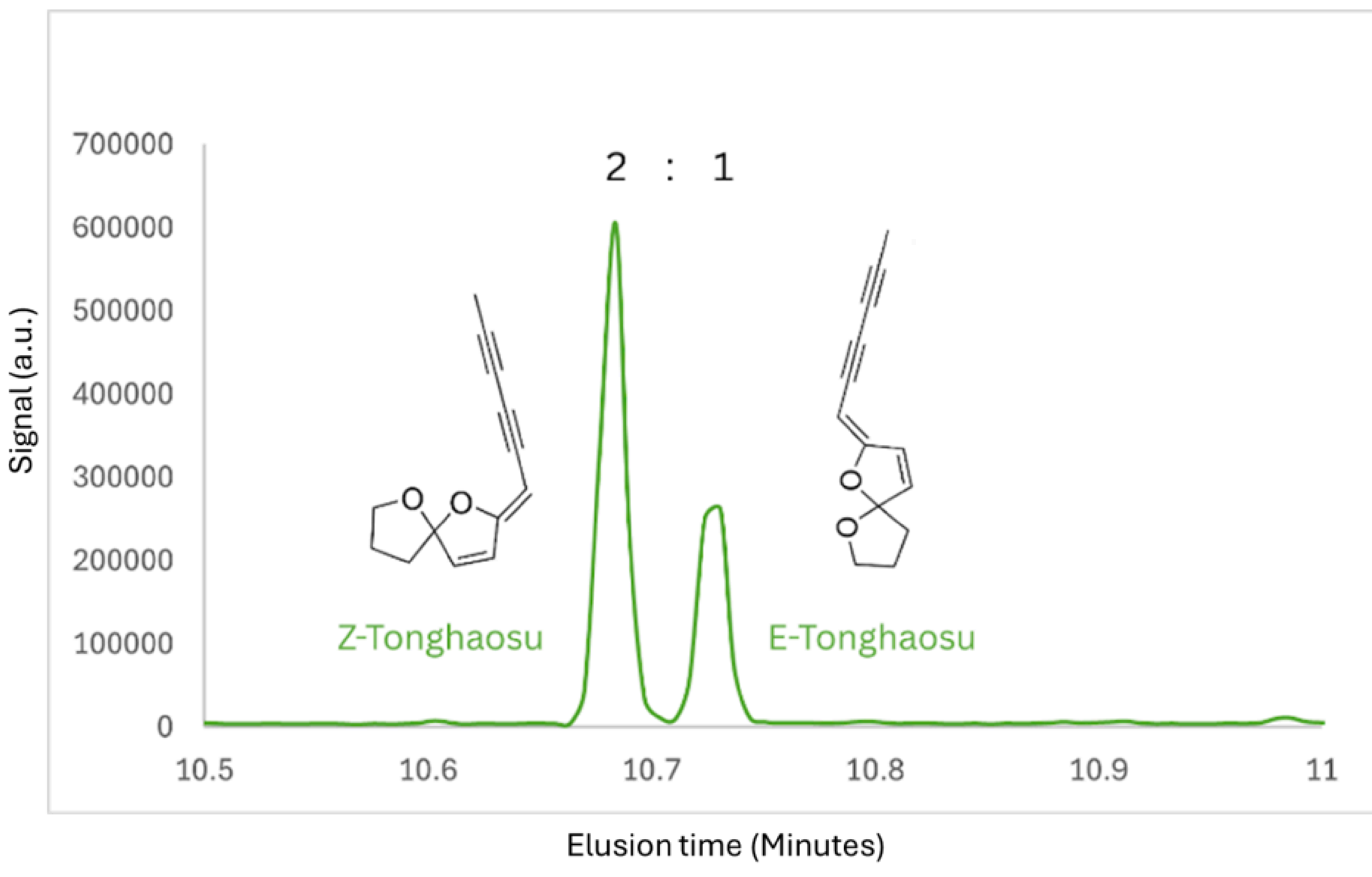

Figure 4. GC-MS chromatograms for *Matricaria discoidea* hydrosol extract, zooming in to the two Tonghaosu isomers.

leaves and flowers individually, it is evident that the flowers have a greater relative abundance of Z-tonghaosu. In contrast, the leaf extract had an even ratio of around 14% per isomer with a greater percentage of E-tonghaosu than found in the flowers.

In contrast to the acetonitrile extract, the hydrosol presented only tonghaosu as observable compounds, Figure 4 and supercritical CO_2 extractions, tonghaosu still appears as the most prevalent compound.

Antifeedant Properties

Originally identified to be the major component responsible for the tanghao plants' antifeedant capabilities, deterring insects while avoiding toxins, tanghaosu presents a potential development of natural or non-toxic insect repellents and insecticides. Thus, its large chemical presence in a plant as common as pineapple weed provides the potential for future development. As previously mentioned, the Z isomer of tonghaosu has been found to have greater antifeedant activity. Thus, as shown through all 6 extracts, Z-tonghaosu is the more prominent compound in pineapple weed, supporting insect-repelling applications through pineapple weed specifically. This apparent abundance also potentially supports recognized applications such as the previously mentioned rubbing, burning, and spraying to repel insects alongside the predator-repelling properties of aromatic terpenes.

Looking specifically at the results of the pineapple weed hydrosol, the GC-MS only revealed tonghaosu as a significant compound and thus represents a promising opportunity for isolated experimentation. The tonhaosu was quantified 3.6 g of Z-tonghaosu and 1.8 g of E-tonghaosu in the initial 250 g sample of pineapple weed. The use of water for the extraction permits a broad potential for applications. The potential insect-repelling properties of tonghaosu may be easily utilized through at-home distillation, only requiring simple kitchenware to extract a hydrosol from the plant.

Conclusion

This study was able to successfully investigate the chemical composition of the common *Matricaria discoidea* under different extraction conditions by GC-MS analysis. Tonghaosu was the major component in its Z and E isomeric forms, with Z-tonghaosu having an earlier elution and greater abundance in all analyzed segments of the plant. This provides support for traditional uses of pineapple weed as an insect repellent and for potential further application as an insecticide, with tonghaosu being a recognized antifeedant. Particularly, the isolation and abundance of tonghaosu, with emphasis on the Z-isomer through a hydrosol, present a promising source for further investigation. In summary, *Matricaria discoidea*'s rich chemical composition provides a favorable resource for the insecticide industry and therapeutic applications.

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