QUARTERLY REPORT

April 1, 2026 – June 30, 2026

Kentucky Tobacco
Research & Development Center

Martin-Gatton
College of Agriculture,
Food and Environment

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MEMORANDUM

DATE: July 31, 2026

TO: Kentucky Tobacco Research Board Members
Legislative Research Commission

FROM: Dr. Ling Yuan
Managing Director, KTRDC

SUBJECT: Kentucky Tobacco Research & Development Center
Quarterly Report for April 1, 2026 – June 30, 2026

Enclosed is a copy of the Kentucky Tobacco Research & Development Center's Quarterly Report for April 1, 2026 – June 30, 2026.

If you have any questions, please feel welcome to contact me at (859) 257-5798 or email lyuan3@uky.edu.

Enc.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Introduction

The legislation (KRS 248.510 - 248.580) which provides funds in support of the research programs at the Kentucky Tobacco Research and Development Center (KTRDC) requires that a quarterly research report be submitted to the Kentucky Tobacco Research Board (KTRB) and the Legislative Research Commission.

The overall reporting plan is:

January 1	-	March 31:	Selected topics
April 1	-	June 30:	Selected topics
July 1	-	September 30:	Selected topics
October 1	-	December 31:	Annual comprehensive report

As required by KRS 248.570, a financial report covering expenditures for the relevant proportion of the July 1, 2025 – June 30, 2026 fiscal year is included in this report.

The news and research publications provided in this quarterly report are a representative selection of KTRDC's output. For a full description of all KTRDC research and activities please refer to the KTRDC Annual Report.

Quarterly News

- The emphasis in KTRDC's research work continues to be on harm reduction and pathogen studies. There is a great deal of collaboration between field and molecular researchers, where the molecular biologists attempt to explain field observations at the cellular level. Examples of these collaborations are:
  - Collaboration with Altria for frog eye leaf spot. The project integrates disease phenotyping with modern genomics to provide sustainable, long-term solutions for tobacco disease management and improved leaf quality.
    - This proposal aims to identify tobacco lines with natural tolerance or resistance to frog-eye leaf spot (FLS), a disease that has become an increasing threat to burley tobacco production in Kentucky.
    - The project will use a greenhouse-based screening assay to evaluate diverse burley, dark tobacco, and wild *Nicotiana* germplasm for resistance.
    - In parallel, comparative transcriptomic analysis of resistant and susceptible lines will be conducted to identify genes and molecular markers associated with FLS resistance. These markers will be validated

and incorporated into marker-assisted breeding to accelerate the development of resistant cultivars.

- There has been a significant amount of work on tobacco terpenes, such as the alpha-cembratriene-4,6-diols (CBT-diols) and cis-abienols, and sugar esters. These compounds are of interest because of their effect on tobacco flavor and aroma, and have been shown to possess insecticidal, antifungal and antibacterial properties. Molecular scientists are now working on the regulation of terpene and sugar ester formation.
- Production of ultra-low alkaloid leaf (novel gene plus *nic1 nic2* mutants) for a small manufacturing company.
- One field project on Tobacco-Specific Nitrosamine (TSNA) reduction.
  - A project on TSNAs and green burley. In addition to the agronomic and breeding plots on the university farm, three of these lines are being tested on a farm.
- A large part of the KTRDC research program, both field and molecular, is devoted to nicotine and nornicotine, particularly lowering these alkaloids in plants.
  - Field and molecular scientists are collaborating to try to understand why the low-alkaloid tobacco lines have such poor leaf quality. It seems that the buildup of nicotine precursors may play a role.
  - Molecular scientists are currently using single cell technology to understand nicotine biosynthesis at the cellular level, and to identify new factors involved in nicotine biosynthesis.

- Activity at the farm this quarter involved mainly seedling production and transplanting, and some sampling.
  - Work in April was limited to the care of seedlings in the greenhouse, and servicing and checking equipment for up-coming field operations.
  - May's activities mainly involved transferring seedlings into float trays in a predetermined sequence corresponding to the randomized order of treatments in the field, and transplanting.
    - Transplanting of the tobacco field trials on the university farm was done on May 19.
  - The entire month of June, during the early growth of the crop, was very wet. The crop has grown out well, but after the heavy rains in late June, some parts of the field were very waterlogged. This has resulted in drowning in

some plots, with plants severely wilted and unlikely to recover and this will impact the reliability of the yield, quality and chemistry results.
    - There was an unprecedented high incidence of tomato spotted wilt virus (TSWV) in our research crop from very soon after setting.
    - The on-farm green burley was transplanted in Bourbon County in mid-June
    - A small sampling for seed screening was done on June 30.
- The June board meeting was held in-person in the McCuiston Conference Room in KTRDC and by Zoom on June 15th 2026.
    - Dr. Ruth McNees of KTRDC gave a presentation entitled "Analytical Capabilities Available at KTRDC".
        - She gave an overview of the collaborations within the University of Kentucky and funding opportunities from USDA and FDA.
        - The analytical laboratory participates in a variety of international collaborative studies with CORESTA, including the ongoing nicotine pouch study and development of the upcoming e-vapor collaborative study.
        - Because the analytical laboratory has the specialized equipment needed for smoking cigars and next generation aerosol analysis of heated tobacco products and e-vapor products, the laboratory has signed up to participate in the upcoming universal adaptor project with the Ohio State University collaborative agreement with FDA.
        - A brief summary of the types of samples that can be analyzed and the instruments used for analysis was provided. Additionally, the 2022 Kentucky Agricultural Census Data was discussed as possible options for what sample analysis would best benefit the Kentucky farmers.
    - Dr. Yuan gave an update on the budget.
        - He noted that there has been a reduction in tax income and that it has been trending downward for at least the past several years, but the last couple of years have been consistent with those in the past.
        - He provided the major job responsibilities for the newly developed field research position.
        - He also provided a summary of the funds available for KTRDC that do not come from the state income tax on cigarettes.
- The calls for papers for the two major conferences have gone out.

- Abstracts were submitted in May by several KTRDC scientists that will attend the CORESTA Congress to be held October 25th – 29th in Victoria Falls, Zimbabwe. The congress theme is "Science for Sustainability and Harm Reduction in the Transforming Tobacco Landscape".
- Abstracts were submitted in May by several KTRDC scientists that will attend the 79th TSRC conference. The conference will be held September 20th – 23rd in Charlotte, NC. The theme is "The Evolving Landscape of Tobacco Harm Reduction."

- The CTRP proficiency testing (PT) program currently covers the certified 1R6F reference cigarette and the certified reference smokeless tobacco products.
  - The current PT rounds are:
    - CIG-2026A – The parameters for this round of testing include smoking parameters o-toluidine, 2,6-dimethylanilin, o-anisidine, 1-aminonaphthalene, 2-aminonaphthalene, 3-aminobiphenyl, 4-aminobiphenyl, Total Particulate Matter (TPM), Puff Count, Benzo[α]pyrene (BaP), BaP-Total Particulate Matter (BaP-TPM) and BaP-Puff Count using the 1R6F reference cigarette as proficiency test material, smoked in both Non-Intense and Intense smoking regimes. This round of testing opened in January 2026 and the data portal for participants to upload data closed in April 2026. The interim report was released for participant review in early May 2026 and the final report was released for participant review in late May 2026.
    - CIG-2026B – The parameters for this round of testing include smoking parameters Ammonia, Acrylonitrile, Isoprene, Benzene, Toluene, 1,3 – Butadiene, Total Particulate Matter, and Puff Count using the 1R6F reference cigarette as proficiency test material, smoked in both Non-Intense and Intense smoking regimes. This round of testing opened in March 2026 and the deadline for data submission was extended from June 2026 to July 2026.
    - CIG-2026C – The parameters for this round of testing include smoking parameters Formaldehyde, Acetaldehyde, Acetone, Acrolein, Propionaldehyde, Crotonaldehyde, 2-butanone, n-butyraldehyde, TPM and Puff Count using the 1R6F reference cigarette as proficiency test material, smoked in both the non-intense and intense smoking regimes. This test also includes the determination of physical properties of the test material, namely: Cigarette Resistance to Draw (pressure drop open)

Cigarette Resistance to Draw (pressure drop closed), Filter Pressure Drop (fully encapsulated), Total Ventilation, Filter Ventilation, Tobacco Weight, Cigarette Weight, Air Permeability, Firmness, Circumference, Cigarette Length, Filter Plug Length, and Tipping Paper Length. This round of testing opened June 4th, and the data submission portal will open July 9th. The interim report is expected to be released in September and the final report is expected to be released in October.

I would like to thank Mr. Matthew Craft, Ms. Anne Fisher, and Dr. Sitakanta Pattanaik for their help with writing some sections of this report, and Mr. Matthew Craft and Ms. Cortney Berry for their help with the compilation.

The KTRDC Quarterly Reports include a copy and brief summary of work published by KTRDC scientists and scientists partly supported by KTRDC.

Report #1 "Natural Tobacco Flavor Profiling Under Heat-Not-Burn Conditions Using Reference Tobacco Leaves and Tobacco from 1R6F Reference Cigarettes".

Antoaneta Mihaylova-Kroumova, Victor Korenkov, and George Wagner

This research explores the flavor compounds released when tobacco is thermally heated to 200–325 °C. Samples analyzed include the filler from 1R6F reference filler and four straight grade tobacco types. The results identified a variety of compounds common to the tobacco types tested, and some that were detected only in a single type of tobacco. The results of this study have the potential to expand on the flavor profile of heated tobacco products.

Unlike combustion processes (> 600 °C), which generate numerous undesirable substances, the heat-not-burn approach (< 350 °C) helps preserve the integrity of natural terpenoids, alkaloids, phenols, and other bioactive molecules while reducing harmful byproducts. This research studied which natural flavor compounds are released from different tobacco types when heated at temperatures typical of heated tobacco products (HTPs) and how these compounds likely contribute to the flavor and aroma profiles. No preliminary modifications were made to the tobacco samples. Samples analyzed include the filler from reference 1R6F cigarettes and four tobacco types were selected: flue-cured, Oriental, Burley, and dark air-cured. Gas Chromatography–Mass Spectrometry (GC-MS) analysis was performed using a Thermal Separation Probe (TSP), which thermally desorbed volatile and semi-volatile constituents from the ground leaf materials at controlled inlet temperatures (200–325 °C). The released compounds were directly introduced into the GC inlet for chromatographic separation and subsequent mass spectrometric identification. Among the numerous peaks detected, flavor-like compounds previously identified in the literature were selected for further evaluation. Flue-cured tobacco yielded 16 identified flavor compounds, oriental tobacco 21, burley 15, and dark air-cured 15. Sixteen compounds were detected in the reference 1R6F cigarette sample. In total, 32 natural flavor compounds were identified across the five tobacco samples. Compounds were also categorized by chemical class and compared across the five tobacco types. Based on this class-wise distribution, the natural organoleptic profile of each tobacco type was estimated.

Report #2 "Exploring the Potential of *Artemisia Annua* for Therapeutic Uses in Heated Tobacco Devices"

Antoaneta Mihaylova-Kroumova, Victor Korenkov, George Wagner & Jeffrey Kinney

This research focuses on the possible application of heated tobacco products devices for use in delivering therapeutic compounds in Artemisia annua. A variety of compounds were identified, with many terpenes that could potentially have medicinal properties. The identification of the terpenes at 100–200 °C show that low thermal release from Artemisia annua could be delivered by a heated tobacco product device. Increasing the temperature to over 275 °C resulted in the identification of additional compounds, many non-terpene and believed to be degradation compounds.

Artemisia annua (A. annua) herb is renowned for its potent antimalarial and broader biological activities. This study aims to explore the potential of *A. annua* for adding therapeutic compounds to low-temperature heated, tobacco-related products. The volatile compounds were released at four different temperatures (100°C to 325°C) and were analyzed using a Thermal Separation Probe (TSP) coupled with GC-MS. A diverse array of compounds was found, with up to 20 different terpenes identified at certain temperatures. Common terpenes found in dry and fresh tissue included Camphene, β-Sabinene, Eucalyptol, γ-Terpinene, Neophytadiene, and Deoxyartemisinin. The potential use of *A. annua* in low-heated tobacco devices for therapeutic advantage is thus considered highly plausible. These findings suggest that *A. annua* could be an effective component in heated tobacco devices, herbal cigarettes, or e-liquids, offering both recreational and medicinal benefits. The terpenes from dry powder and fresh leaf, could enhance the therapeutic value and aromatic qualities of products. Additionally, the ability to release these beneficial compounds at temperatures as low as 100°C provides an opportunity to optimize the efficiency and safety of such devices by reducing the need for higher operating temperatures, which can lead to the formation of harmful by-products.

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Natural Tobacco Flavor Profiling Under Heat-Not-Burn Conditions Using Reference Tobacco Leaves and Tobacco from 1R6F Reference Cigarettes *

by

Antoaneta Mihaylova-Kroumova [1], *Victor Korenkov, and George Wagner*

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SUMMARY

Studying the organoleptic properties of heated tobaccos in relation to natural flavors is essential for understanding how lower-temperature processing influences the release of intrinsic tobacco flavor compounds. Unlike combustion processes (> 600 °C), which generate numerous undesirable substances, the heat-not-burn approach (< 350 °C) helps preserve the integrity of natural terpenoids, alkaloids, phenols, and other bioactive molecules while reducing harmful byproducts. This research studied which natural flavor compounds are released from different tobacco types when heated at temperatures typical of heated tobacco products (HTPs) and how these compounds likely contribute to the flavor and aroma profiles. No preliminary modifications were made to the tobacco samples. Four tobacco types were selected: flue-cured, Oriental, Burley, and dark air-cured. The ground lamina of these tobaccos, along with filler from reference 1R6F cigarettes (all provided by the Center for Tobacco Reference Products), were analyzed. Gas Chromatography–Mass Spectrometry (GC-MS) analysis was performed using a Thermal Separation Probe (TSP), which thermally desorbed volatile and semi-volatile constituents from the ground leaf materials at controlled inlet temperatures (200–325 °C). The released compounds were directly introduced into the GC inlet for chromatographic separation and subsequent mass spectrometric identification. Among the numerous peaks detected, flavor-like compounds previously identified in the literature were selected for further evaluation. Flue-cured tobacco yielded 16 identified flavor compounds, oriental tobacco 21, burley 15, and dark air-cured 15. Sixteen compounds were detected in the reference 1R6F cigarette sample. In total, 32 natural flavor compounds were identified across the five tobacco samples. Flavor properties of each compound were assigned based on various literature sources. Compounds were also categorized by chemical class and compared across the five tobacco types. Based on this class-wise distribution, the natural organoleptic profile of each tobacco type was estimated. Several natural compounds were detected that have not previously been reported in aerosols generated from heated tobacco products at low temperatures (200–325°C). These compounds may potentially expand the flavor profile of heated tobacco products. [Contrib. Tob. Nicotine Res. 35 (2026) 58–67]

KEYWORDS:

Thermal separation probe; tobacco natural flavors; GC-MS; heat-not-burn tobacco; research tobaccos.

ZUSAMMENFASSUNG

Die Untersuchung der organoleptischen Eigenschaften erhitzter Tabake im Zusammenhang mit natürlichen Aromen ist wichtig, um zu verstehen, wie die Verarbeitung bei

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niedrigeren Temperaturen die Freisetzung intrinsischer Tabakaromastoffe beeinflusst. Im Gegensatz zu Verbrennungsprozessen (> 600 °C), bei denen zahlreiche unerwünschte Stoffe entstehen, trägt der Heat-not-Burn-Ansatz (< 350 °C) dazu bei, die Integrität natürlicher Terpenoide, Alkaloide, Phenole und anderer bioaktiver Moleküle zu erhalten und gleichzeitig schädliche Nebenprodukte zu reduzieren. In dieser Arbeit wurde untersucht, welche natürlichen Aromastoffe aus verschiedenen Tabakarten freigesetzt werden, wenn sie bei Temperaturen erhitzt werden, die für Heated Tobacco Products (HTPs) typisch sind und wie diese Stoffe wahrscheinlich zu Geschmacks- und Aromaprofilen beitragen. Die Tabakproben wurden nicht vorbehandelt oder verändert. Vier Tabaktypen wurden ausgewählt: Flue-Cured, Oriental, Burley und Dark Air-Cured. Die gemahlene Lamina dieser Tabake sowie der Filler von Referenzzigaretten 1R6F (alle bereitgestellt vom Center for Tobacco Reference Products) wurden analysiert. Die Gaschromatographie–Massenspektrometrie (GC-MS) wurde mit einer Thermal Separation-Sonde durchgeführt, die flüchtige und halbflüchtige Bestandteile aus dem gemahlenen Blattmaterial bei kontrollierten Inlettemperaturen (200–325 °C) thermisch desorbierte. Die freigesetzten Verbindungen wurden direkt in das GC-Inlet eingebracht, dort chromatographisch getrennt und anschließend massenspektrometrisch identifiziert. Unter den zahlreichen detektierten Peaks wurden aromarelevante Verbindungen, die in der Literatur bereits beschrieben sind, für eine weitere Auswertung ausgewählt. Im Flue-Cured-Tabak wurden 16 Aromastoffe identifiziert, im Oriental-Tabak 21, im Burley-Tabak 15 und im Dark Air-Cured-Tabak ebenfalls 15. In der Referenzzigarette 1R6F wurden 16 Verbindungen gefunden. Insgesamt wurden 32 natürliche Aromastoffe in den fünf Tabakproben identifiziert. Die Geschmackseigenschaften jeder Verbindung wurden anhand verschiedener Literaturquellen zugeordnet. Die Verbindungen wurden zudem nach chemischen Klassen kategorisiert und über die fünf Tabakarten hinweg verglichen. Auf Grundlage dieser klassenweisen Verteilung wurde das natürliche organoleptische Profil jedes Tabaktyps abgeschätzt. Mehrere natürliche Verbindungen wurden nachgewiesen, über die bisher nicht in Aerosolen von erhitzten Tabakprodukten bei niedrigen Temperaturen (200–325 °C) berichtet wurde. Diese Stoffe könnten das Aromaprofil von Heated Tobacco Products erweitern. [Contrib. Tob. Nicotine Res. 35 (2026) 58–67]

RESUME

L'étude des propriétés organoleptiques des tabacs chauffés en relation avec les arômes naturels est essentielle pour comprendre comment un traitement à plus basse température influence la libération des composés aromatiques intrinsèques du tabac. Contrairement aux processus de combustion (> 600 °C), qui génèrent de nombreuses substances indésirables, l'approche « heat-not-burn » (< 350 °C) permet de préserver l'intégrité des terpènoïdes, alcaloïdes, phénols et autres molécules bioactives naturelles, tout en réduisant les sous-produits nocifsCette recherche a étudié quels composés aromatiques naturels sont libérés à partir de différents types de tabac lorsqu'ils sont chauffés à des températures typiques des produits du tabac chauffé (HTP), et comment ces composés contribuent probablement aux profils d'arôme et de saveur. Aucune modification préalable n'a été apportée aux échantillons de tabac. Quatre types de tabac ont été sélectionnés: flue-cured, oriental, burley et dark air-cured. La lamina broyée de ces tabacs, ainsi que le remplissage de cigarettes de référence 1R6F (tous fournis par le Center for Tobacco Reference Products), ont été analysés. L'analyse par chromatographie en phase gazeuse–spectrométrie de masse (GC-MS) a été réalisée à l'aide d'une sonde de séparation thermique (TSP), qui a désorbé thermiquement les constituants volatils et semi-volatils du matériau foliaire broyé à des températures d'injection contrôlées (200–325 °C). Les composés libérés ont été introduits directement dans l'injecteur du GC pour la séparation chromatographique, puis identifiés par spectrométrie de masse. Parmi les nombreux pics détectés, des composés de type aromatique déjà décrits dans la littérature ont été sélectionnés pour une évaluation plus approfondie. Le tabac flue-cured a donné 16 composés aromatiques identifiés, le tabac Oriental 21, le Burley 15 et le dark air-cured 15. Seize composés ont été détectés dans l'échantillon de cigarette de référence 1R6F. Au total, 32 composés aromatiques naturels ont été identifiés dans les cinq échantillons de tabac. Les propriétés gustatives de chaque composé ont été attribuées sur la base de diverses sources bibliographiques. Les composés ont également été classés par classe chimique et comparés entre les cinq types de tabac. Sur la base de cette répartition par classes, le profil organoleptique naturel de chaque type de tabac a été estimé. Plusieurs composés naturels ont été détectés qui n'avaient pas encore été signalés dans des aérosols de produits du tabac chauffé à basse température (200–325 °C). Ces composés pourraient élargir le profil aromatique des produits du tabac chauffé. [Contrib. Tob. Nicotine Res. 35 (2026) 58–67]

INTRODUCTION

In recent years, heated tobacco products (HTPs) have emerged as a significant innovation in the tobacco and nicotine markets. Unlike conventional cigarettes that burn tobacco at high temperatures, HTPs are designed to heat tobacco to lower temperatures (typically below 350 °C), producing an inhalable aerosol without pyrolysis (1). This process reduces or avoids the formation of many harmful and potentially harmful constituents (HPHCs) associated with tobacco pyrolysis, offering an alternative experience that is closer to traditional smoking than e-cigarettes, but potentially with lower toxicant exposure (2).

The commercial success of products like 'IQOS' (Philip Morris International), 'glo' (British American Tobacco), and 'Ploom' (Japan Tobacco International) has driven rapid global market expansion of HTPs (3). These products appeal particularly to adult smokers seeking alternatives to cigarettes, contributing to a shift in consumer preferences and public health discussions. Regulatory authorities and scientific bodies have responded with increased research into their chemical emissions, health effects, and population-level impacts (4).

A distinctive sensory characteristic of HTPs is their

relatively mild flavors, which mainly arise from the gentle thermal release of compounds naturally intrinsic to tobacco leaves. However, pure tobacco without added flavorings or extensive casing produces a flavor profile, often described as woody, slightly nutty, faintly leathery, and mildly sweet (5). Heating tobacco at controlled temperatures preserves these delicate notes, while minimizing the formation of harsh, burnt flavors typically associated with cigarette smoke (5, 6). Additionally, the use of humectants like glycerol can influence the aerosol's mouthfeel, contributing to a smoother sensory experience (7, 8).

Despite the potential for reduced exposure, debates continue regarding the long-term health risks of HTPs. Some regulatory agencies, such as the U.S. Food and Drug Administration (FDA), have authorized the marketing of certain HTPs as modified risk tobacco products (MRTPs) (2), but cautioning that these products are not safe and that complete cessation remains the best choice for health (4). Overall, heated tobacco products represent an evolving landscape of nicotine delivery technology, reflecting both technological innovation and public health challenges in the 21st century.

The natural flavor of tobacco is derived from a complex group of volatile organic compounds synthesized through the plant's endogenous biochemical pathways and subsequently transformed during curing and fermentation. Research has demonstrated that terpenoids, alkaloids, and phenolic compounds interact synergistically to establish the distinctive sensory profile of tobacco (9). These intrinsic molecules not only define the organoleptic properties of the product but also function as chemical markers for the evaluation of tobacco quality and authenticity. The elucidation of these interactions underscores the convergence of traditional agricultural practices and modern analytical methodologies in advancing the scientific understanding of tobacco flavor chemistry (9).

Factors influencing the flavor profile of tobacco

The flavor profile of tobacco is influenced by several key factors, including the tobacco variety and the curing process employed after harvest.

Tobacco varieties: Different tobacco varieties contribute distinct natural flavor characteristics due to genetic and biochemical variations: Virginia (flue-cured) tobacco possesses natural sweetness with honey-like and citrus undertones, primarily attributed to high sugar content (10), Burley tobacco exhibits a more earthy and nutty flavor with lower natural sweetness, reflecting its low sugar and high alkaloid content. Oriental tobacco is characterized by spicy, aromatic, and floral notes, owing to its smaller leaf size and higher concentrations of volatile aromatic compounds (9, 10).

The methods of curing tobacco leaves after harvest are different depending on the kind of tobacco, and significantly modify the chemical composition and, consequently, the flavor. Air curing is a process of slow drying in well-ventilated barns and produces mild, earthy, and low-sugar flavor profiles (10). Fire curing (exposure to open wood fires) introduces smoky, robust, and heavily aromatic flavors, enriching the tobacco with smoke-derived phenolic compounds (9).

Sun-curing under direct sunlight exposure yields lighter, aromatic flavors by promoting natural enzymatic reactions at low temperatures (9). Flue-curing process in controlled environments enhances sweetness and develops bright, flavorful notes, primarily by preserving natural sugars while reducing green leaf bitterness (10, 11).

These factors collectively determine the sensory characteristics of tobacco products, influencing their application in cigarettes, cigars, and pipe blends. The organoleptic impact of chemical composition and processing factors is most established in the context of smoked tobacco (e.g., cigarettes, cigars, pipes) (7, 11). The key aroma compounds in tobacco and their sensory properties are given in several publications (12, 14, 15).

Heated tobacco and smokeless products are studied with similar frameworks, but data is more recent and evolving. While many flavor-affecting principles are shared with smoked tobacco, HTPs rely more on: thermal degradation (not combustion), glycerol-mediated transport, and volatile precursor chemistry. This results in smoother, less harsh and more aroma-focused profiles, but also introduces more variability depending on device design and tobacco formulation (6, 7, 12, 13).

The moderate temperatures employed in HTPs help preserve subtle flavor notes, such as woody, nutty, floral, and slightly sweet aromas, which are often diminished by combustion. Although the flavor derived from pure tobacco is relatively restrained compared to flavored or additive-enhanced products, natural volatiles like alcohols, esters, aldehydes, and ketones play a central role in defining the aerosol's aroma and taste. Moreover, microbial fermentation during curing further diversifies the flavor composition (9, 11).

Heated tobacco products (HTPs) operate at lower-than-burning temperatures (typically below 350 °C), causing tobacco flavor deliverance while avoiding the generation of combustion byproducts. Studies have demonstrated that the heat-not-burn process preserves a greater proportion of the natural volatiles compared to conventional smoking, allowing for a milder, less acrid flavor profile (9, 16). Characterization of aerosols from HTPs has revealed the presence of flavor-active compounds such as β-damascenone, megastigmatrienone, furans, and pyrazines, which contribute to floral, sweet, woody, and nutty notes (8, 16).

Several studies have documented volatile and semi-volatile flavor-related compounds generated under heat-not-burn conditions (6–8, 12, 16). These works provide specific compound lists based on the analytical methods employed in each study.

The aim of this study was to identify and characterize the volatile compounds using a thermal separation probe, which enables direct thermal release from the tobacco matrix without preliminary sample derivatization, extraction, or concentration. The composition of volatile and semi-volatile flavor compounds obtained through direct thermal detachment and volatilization from the tobacco matrix has not been previously reported. The study investigated which natural flavor compounds are released from different tobacco types when heated at temperatures typical of HTPs and how these compounds contribute to the flavor and aroma profiles.

MATERIAL AND METHODS

Research ground tobaccos and tobacco from reference cigarettes were purchased from the Center for Tobacco Reference Products (17), University of KY, Lexington, USA. Research tobaccos included: RT2 Ground Flue-Cured Tobacco; RT3 Ground Oriental Tobacco; RT4 Ground Burley; RT9 DAC Ground Dark Air-Cured Tobacco. Each of those reference tobaccos represented a blend of different grades with each grade coming from any country in the world where they grow that tobacco (C. Fisher, personal communication).

1R6F reference cigarettes

The tobacco from reference cigarettes has a long history of use in the cigarette industry as quality control monitors, model cigarette systems, and analytical method development tools. The filler of 1R6F reference cigarettes contain the following materials: flue-cured 34%; Burley 24%; Oriental 12%; reconstituted 20% (by products of stemming); expanded flue-cured 7%; expanded Burley 3%; Glycerin ~1.7% dry weight basis, propylene glycol (PG) 1%, and Isosweet ~ 6.3%. Isosweet refers to a humectant (high fructose corn syrup) used in the specific blend of the 1R6F Kentucky reference cigarette. It is not a standard commercial product name on its own, but a specific measurement within a tobacco product formulation (17).
The fillers of five cigarettes were grounded to 1 mm-size particles using a Thomas Scientific Wiley Mill (Swedesboro, NJ, USA) before further processing.
Samples were stored at −20 °C. They were conditioned at controlled temperature (22 ± 1 °C) and relative humidity (60 ± 5%) for at least 24 h prior to analysis to ensure moisture equilibrium. There was no extraction or derivatization of samples. Tobacco samples were heated under controlled temperatures simulating typical HTP operating temperatures (200–325 °C).

METHODS

GC-MS were used to access each of the four research ground tobaccos and the filler blend from the 1R6F reference cigarettes for each of the three defined temperatures. Thermal separation probe (TSP) (Agilent, Santa Clara, CA, USA) is an adapter connected to the inlet of the gas chromatography (GC) column, into which a micro vial containing a sample is inserted. TSP applies precise temperature without combustion. TSP utilizes the GC multimode inlet to introduce the sample into the gas stream. The inlet split mode minimizes column and detector overload as well as detector contamination (18).
The inlet and the GC column have independent temperature programming. Only the compounds vaporized at a specific inlet temperature are carried by the helium gas into the column and the detector. Non-vaporized compounds with high boiling points remain inside the micro vials and can be discarded after each run (18).
No preliminary sample processing, extraction, purification, or derivatization was applied. Cured plant material was directly analyzed. The leaf material (5 mg) was placed into an ultra inert micro vial (Agilent Part No: 5190-3187) which was then inserted into a TSP adapter (Agilent Part No: G4382-6300). The TSP was introduced into the Multimode Inlet (MMI). This setup allows for the release of volatile and semi-volatile compounds from the leaf matrix in the TSP inlet before entering the GC column. This method minimizes cross-contamination between samples (18).
The following instrumentation was used in the experiments: Gas Chromatograph: Agilent 8890 GC System; Mass Spectrometer: Agilent 5977B GC MSD; Software: Agilent Mass Hunter (Agilent Technologies) for GC-MS (19).
The operational conditions were based on the application note for the analysis of alcohols (20). Agilent application notes are technical documents that describe practical use cases, methods, and performance evaluations for Agilent's instruments. Gas chromatography was performed using an HP-5MS UI non-polar column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). Helium (99.9% purity) was used as the carrier gas at a constant flow rate of 1.2 $mL \cdot min^{-1}$, with the initial temperature set at 50 °C. The oven temperature program was as follows: an initial temperature of 50 °C held for 1 min, followed by a ramp of 3.4 $°C \cdot min^{-1}$ to 230 °C, where it was held for 6 min, resulting in a total run time of 59.94 min. The inlet was operated in split mode with a split ratio of 40:1. Three separate inlet temperatures (200 °C, 275 °C, and 325 °C) were applied for each tobacco sample. Separate experiments were carried out for each tobacco sample, with three replicates at 200 °C, three at 275 °C, and three at 325 °C. Mass spectrometry (MS) parameters were as follows: the ion source temperature was set to 230 °C, and the interface temperature was maintained within a range of 250 °C to 320 °C. Electron ionization (EI) was used as the ionization mode, with an electron energy of 70 eV. The quadrupole temperature was held at 150 °C. Mass spectra were acquired in scan mode over a mass range of 30.00 to 550.00 m/z, with a scan rate of 2.8 scans per second. An electron multiplier was used as the detector. The column HP5 UI is a nonpolar one. It retains compounds mainly based on boiling point. Highly volatile compounds elute within a few minutes. Moderately volatile compounds may elute in 10–30 min (e.g., fatty acid methyl esters, terpenes). Semi-volatile compounds (e.g., steroids, alkaloids) often elute after 30 min. However, the compounds are still volatile enough to be analyzed by GC-MS, meaning they have a boiling point low enough for thermal desorption and vaporization in the injector (21).

DATA PROCESSING

In the current study, a non-targeted analysis (NTA) of chemicals in tobacco was applied. NTA aims to identify a wide range of chemicals present, without specifically targeting a limited set. This contrasts with targeted analysis, where a small, predefined set of chemicals is the focus (22).
For each temperature at least a hundred compounds were detected per run. Total Ion Chromatograms (TICs) were obtained in triplicate for each temperature (200 °C, 275 °C, or 325 °C). In order to chemically identify hundreds of compounds from each of the chromatographic runs we applied a rapid search of mass spectral library. This search

presented Probability Based Matching (PBM) algorithm. The match score is a mathematical calculation, often based on a modified cosine similarity, that compares the presence and relative intensity of the ion fragments on the sample's mass spectrum to those in the library's known compound spectrum. The match score is expressed in percentage. An 80% match is considered good with moderate reliability, but it does not guarantee accuracy (23). Only compounds having a quality score of ≥80% were selected. Further identification of the selected compounds was carried out using the National Institute of Standards and Technology (NIST) Mass Spectral Library, version 17.1. The screening of compounds from the three repeats was performed using the R-project for statistical computing (24). The constituents having a quality score ≥80% in at least two out of three replicates were selected and processed further. This process was repeated for each of the three temperatures (200 °C, 275 °C, and 325 °C). The abundance of peaks was measured as signal intensity or abundance of the detected ions. There was a problem using obtainable external standards for confirmation of some compounds when added to the samples before TSP volatilization. The standards' retention times and abundance were different, when applied separately, and when added to the tobacco powder. Due to the specificity of the method, the matrix effect may play a big role in volatilization. The presence of D-Limonene/Limonene was observed at 325 °C in most of the analyzed tobacco samples. Limonene standard itself was detected very early on the chromatograms at 100 °C, but was degraded at >100 °C (not published). In contrast, limonene from tobacco was observed only at >300 ºC. Similarly standard limonene mixed with tobacco material showed only at 325 °C (not published). Similar discrepancy was observed with other external standards, like 3-methylpentanoic acid (not published). For these reasons, external standards were not used, and identification was on the basis of PBM and NIST searches. Many of those compounds although natural products were not identified as organoleptic in the literature and were excluded from the list. Other chemicals were also natural products, but they have not been found in tobacco leaf, cigar smoke or HTP aerosols (Chat GPT 4o). They were also excluded from the list. Some of the compounds had high percent match when using PBM Quick Search, but they were not found with NIST spectral library search, and they were also excluded. The potential fragrance and flavor value of compounds was assessed using the tobacco 'flavor lists' (12, 27–29).
The data were organized into tables showing the identified chemicals at each of the three temperatures (Supplemental Tables S1–S6). Additionally, the volatiles from studied samples were compared, and their organoleptic potential was estimated.

RESULTS AND DISCUSSION

RT2 Ground Flue-Cured Tobacco

At 200 °C, 25 compounds with ≥80% match were observed; at 275 °C, there were 41, and at 325 °C there were 61 compounds, respectively. The screening of those compounds was conducted as described in the section 'Data processing'. However, many of those compounds although natural products are not thought to possess organoleptic properties so they were excluded. After the screening, a total of 17 compounds were selected. Some were found at all temperatures and some were unique for only one temperature (Supplemental Material, Table S1). The numbers present relative average abundance (peak area × 10^6) and the standard deviation (× 10^6). The most abundant compound was neophytadiene, followed by acetic acid. As seen from Table S1, the standard deviation for most of the selected chemicals was below 20%, but some were above 30%, as for acetate. The later one gave a poorly shaped peak with variable retention, reflecting in high standard deviation. The average percentage standard deviations in non-targeted analysis in the range of 20–30% are suggested as potentially expected or acceptable in certain NTA contexts (30). Similar variations in the standard deviation were found in the other varieties of research tobacco and in the tobacco from reference cigarettes.

RT3 Ground Oriental Tobacco

Compound screening, similar to that done for flue-cured tobacco was used for the Oriental tobacco. Twenty-one compounds were chosen (Supplemental Material, Table S2) according to the Data processing criteria. What is notable is a relatively high abundance of furfural; 2-furanmethanol; 2-furancarboxaldehyde, 5methyl-; and furaneol. These chemicals are products of Maillard reaction, where sugars interact with amino acids to form fragrant compounds. Oriental tobacco is unique in producing sugar esters on the surface of leaves. At higher temperatures sugar esters are degraded to acyl groups and sugar moieties that further are degraded to form reducing sugars (glucose, fructose, lactose, and maltose) involved in Maillard reaction (31, 32). Most of the chemicals were detected only at 275 °C and 325 °C.

RT4 Ground Burley

After the screening of volatile and semi-volatile compounds, 16 flavor and fragrance compounds were chosen (Supplemental Material, Table S3) according to the 'Data processing' criteria. The general organoleptic characteristics of Burley tobacco are earthy and nutty flavors, with less sweetness in the taste. The highest abundances were found for neophytadiene, followed by 2,3`-Dipyridyl and phenol. Most of the compounds were detected at 325 °C. 2,3`-Dipyridyl and phenol together intensify smoky and bitter/harsh undertones**,** especially under thermal exposure (low-temperature pyrolysis), contributing to the drier, harsher end of the tobacco flavor spectrum (11, 33).

RT9 DAC Ground Dark Air-Cured Tobacco

Dark air-cured tobacco has a deep, rich, and fermented organoleptic profile. It is dominated by: phenolics (smoky and leathery tones), nitrogen heterocycles (bitter, dry notes), sweet and spicy volatiles (furans and ketones), fermentation-related acids and esters (tangy, fruity, pungent notes) (11, 34).
In RT9 DAC Ground Dark Air-cured Tobacco leaves 15 organoleptic compounds were selected (Supplemental

Material, Table S4) according to the data processing criteria. The most abundant compounds were neophytadiene and farnesol, followed by furfural and *trans*-geranylgeraniol. Neophytadiene itself is not highly fragrant but contributes to the overall flavor and sensory experience of tobacco and can act as a flavor enhancer (35). Farnesol is a natural compound with a delicate green floral scent and notes of lily of the valley. It is listed as one of the 599 additives in cigarettes in a 1994 report by top cigarette companies (36). In the dark air-cured research tobacco the farnesol is not an additive but naturally found. However, its natural emissions probably are not sufficient to give distinctive flagrance.

Tobacco from 1R6F reference cigarettes

1R6F Reference cigarettes are established currently as major reference cigarettes. Their filler is a combination of flue-cured, Burley, and Oriental types of tobacco, as described in the Materials section. A total of 16 organoleptic compounds were found (Supplemental Material, Table S5) according to the data processing criteria. They prevailed at 275 °C and 325 °C. The most abundant compounds were furfural, 2-furanmethanol, and acetic acid, followed by neophytadiene and trans-geranylgeraniol. Trans-geranylgeraniol is described with woody and herbal aromas, with mild floral and citrus notes (37).

Comparison of research tobaccos and tobacco from 1R6F reference cigarettes' chemical profiles

For each individual tobacco type, as well as for the filler of 1R6F reference cigarettes, the compounds that met the specified criteria were organized as follows: all volatile and semi-volatile compounds detected at the three temperatures were combined into a single column for each sample. The temperatures at which the compounds were released is shown in the respective supplementary tables. The five resulting columns were then aligned. Identical compounds across all samples were placed in the same row to allow direct comparison. The composition of volatile and semi-volatile compounds from all five reference samples was compared, and the organoleptic properties of each compound are shown in Table 1. In the last column of the table, the references to the flavor and/or fragrance were given. A total of 32 natural compounds with organoleptic properties were found according to the data processing criteria. The most ubiquitous volatiles, found in at least four of the five reference products were acetic acid; furfural; 2-Furancarboxaldehyde, 5-methyl-; megastigmatrienone; 2,3`-dipyridyl; neophytadiene; *trans*-geranylgeraniol; and thunbergol. Only four volatiles were common for all reference products.

In order to compare their organoleptic profiles, the compounds were sorted in chemical classes contributing to the aroma and taste (Table 2). Table 2 shows the classifications: Pyranones/lactones prevailed in flue-cured leaves; furan derivatives and ketone/diketones prevailed in Oriental tobacco; diterpenes/diterpenoids prevailed in dark air-cured tobacco.

The five tobacco samples exhibited distinct organoleptic profiles determined by the composition of compound classes (Table 3). The estimation of the natural aroma and the taste of the research tobaccos and tobacco from 1R6F reference cigarettes was made on the basis of the chemical's composition and chemical class profiles, and with the help of Chat GPT4o. It is important to notice that the organoleptic properties were estimated solely on the basis of selected flavor compounds, not on the basis of all compounds found in the chromatograms.

Flue-cured sample was characterized by a high number of pyranones/lactones (four) and balanced terpenoid profile, suggesting a sweet, creamy aroma and mild acidic taste. Oriental tobacco stood out with the highest number of ketones/diketones (four) and furans (four), indicating strong roasted, caramelized, and smoky aroma and taste characteristics. Burley tobacco sample was chemically simpler regarding aroma-related classes (no pyranones or hydroxy ketones), with only one sesquiterpenoid-diol and two ketones, indicating a light and less intense profile. Dark air-cured tobacco contained no pyranones or hydroxy ketones but all major terpene types, implying a more herbal, less sweet profile. Tobacco extracted from1R6F reference cigarettes showed a balanced profile across all compound classes; but had contributions from all aroma/taste classes, suggesting moderate aroma and smooth taste. Previous studies have shown that oxidative aging and storage conditions significantly impact the volatile profile of tobacco products, especially through the breakdown of carotenoids and phenolic precursors (7, 11).

The natural flavor estimation was based on the organoleptic properties of individual compounds, which represents a limitation of this approach. There is a lack of sensory panel evaluation to validate that the estimated aromas and tastes in Table 3 are actually perceived sensorially.

CONCLUSIONS

A total of 32 compounds were detected across all five samples within the temperature range of 200–325°C using the TSP coupled with GC-MS. Of these, fifteen compounds – Acetic acid; Furfural; 2-Furanmethanol (Furfuryl alcohol); 5-Methyl-2-furancarboxaldehyde (5-Methylfurfural); Pyridine; Guaiacol (2-Methoxyphenol); Maltol; 5-Hydroxymethylfurfural (HMF); Phenol; Nicotyrine; Megastigmatrienone; Neophytadiene; Nootkatone; Longifolene; and Farnesol – have been previously identified in the aerosol emissions of HTPs, in tobacco leaf, and in cigarette smoke. (38).

Three additional compounds – 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, solanone, and 2,3'-dipyridyl – have already been reported in low-temperature headspace studies of reference tobaccos and classified as natural flavors (25).

The remaining compounds identified in this study are known constituents of tobacco or products of thermal degradation under combustion or pyrrolic conditions, but their presence in aerosol from HTP has not been specifically confirmed in the literature (38).

In the current study, the authenticity of furaneol; 1,2-cyclohexanedione; D-limonene/Limonene; cyclotene; spiro[3.4]octan-5-one; ethanone; 1-(3-methylphenyl)-; 6-ethyl-5,6-dihydro-2H-pyran-2-one; 5-hydroxymaltol; 2-methoxy-4-vinylphenol; solanone; phenol,2-methoxy; patchoulane;

Table 1. Comparison of volatile and semi-volatile compounds in the five studied samples. The presence or absence of a chemical is indicated by "+" and "—". Numbers in the right most column refer to reference numbers.

CAS	Name	Flue-cured	Oriental (Turkish)	Burley	Dark air-cured	1R6F filler	Organoleptic properties	Ref. numbers
000064-19-7	Acetic acid	+	+	—	+	+	flavor food additive	26
000098-01-1	Furfural	—	+	+	+	+	brown type flavor; aromatic odor reminiscent of almonds	26
000098-00-0	2-Furanmethanol	—	+	—	—	+	burnt type flavor, bready type odor	26
000620-02-0	2-Furancarboxaldehyde, 5-methyl-	+	+	+	+	+	fruity flavor, sweet, and caramel-like	26
010230-62-3	Furaneol	—	+	—	—	—	less sweet, caramel-like aroma	31
000110-86-1	Pyridine	—	+	—	—	+	fishy-type odor	25
000765-87-7	1,2-Cyclohexanedione	—	+	—	—	—	nutty flavor and odor	40
005989-27-5	D-Limonene	+	—	+	+	+	citrus flavor	26
000138-86-3	Limonene	+	—	+	+	+	citrus flavor	26
000765-70-8	Cyclotene	—	+	+	—	—	rich caramel, burnt sugar, or maple syrup-like flavor	25
000090-05-1	Guiacol	—	+	—	—	—	woody flavor, phenolic odor	40
010468-36-7	Spiro[3.4]octan-5-one	—	+	—	—	—	woody, camphoraceous odor	41
000118-71-8	Maltol	+	—	—	—	—	a sweet aroma, of caramel and cotton candy-like	42
000536-90-3	Ethanone, 1-(3-methylphenyl)-	—	+	—	—	—	fruity, sweet, and floral aroma and fragrance	26
028564-83-2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	+	+	—	—	+	sweet, caramel-like notes	26
019895-35-3	6-Ethyl-5,6-dihydro-2H-pyran-2-one	+	—	—	—	—	sweet, caramel-like, coconut, slightly creamy	43
001073-96-7	5-Hydroxymaltol	+	—	—	—	—	sweet, caramel-like, slightly toasty aroma	26
000067-47-0	5-Hydroxymethylfurfural	+	—	—	—	—	mildly sweet, caramel-like	26
007786-61-0	2-Methoxy-4-vinylphenol	—	+	—	—	—	smoky, clove-like, spicy, and slightly sweet	26
054868-48-3	Solanone	—	—	+	—	—	warm, woody, and tobacco-like scent	26
000108-95-2	Phenol	+	—	+	+	—	smoky and tar-like aroma	44
038818-55-2	Megastigmatrienone	+	+	+	+	+	tobacco / insence aroma	25
000581-50-0	2,3'-Dipyridyl	+	+	+	+	+	tobacco taste and flavor improving agent	25
000504-96-1	Neophytadiene	+	+	+	+	+	tobacco flavor enhancer; green, herbal, and mildly sweet aroma	35
004674-50-4	Nootkatone	—	—	—	—	+	scent and flavor of grapefruit	26
025491-20-7	Patchoulane	—	—	+	+	—	mild, earthy, and camphoraceous aroma	26
000475-20-7	Longifolene	—	+	+	+	+	woody, pine-like, resinous scent	26
024034-73-9	*trans*-Geranylgeraniol	+	+	+	+	+	floral, rose-like scent.	26
034318-21-3	Ionol, 3-oxo-	+	+	—	+	—	warm, woody, and floral aroma and flavor	26
000150-86-7	Phytol	—	+	—	+	—	grassy and green notes	45
025269-17-4	Thunbergol	+	+	+	+	+	characteristic, pleasant aroma	43
004602-84-0	Farnesol	—	—	+	+	+	sweet, floral, with a delicate lily-of-the-valley nuance	26

trans-Geranylgeraniol; ionol,-3-oxo, phytol; and thunbergol; was confirmed with PBM and NIST MS searches. The TSP method allowed volatile and semi-volatile compounds to be released directly from untreated tobacco, without extraction or chemical treatment. Some compounds found this way have not been reported before at similar low heating temperatures, suggesting that direct heating can show more of tobacco's natural volatile profile. Although a commercial heated tobacco device was not used, the temperatures matched heat-not-burn conditions, so the results showed which natural flavor compounds can be released during low-temperature heating.

Differences in the compounds released from the five samples showed the effects of tobacco type and how those tobacco types have been processed. These differences may also arise from batch-specific variations in tobacco composition, aging effects, or oxidative degradation that selectively enhance the formation or thermal release of phenolics and ketones. Using odor descriptions from the literature, the tobacco from 1R6F cigarettes released compounds related to a more balanced woody-floral aroma with a comparatively milder sensory profile. The other tobaccos were richer in certain groups such as terpenoids, phenols, or carbonyl compounds.

Table 2. Classification of volatile compounds identified in research tobaccos and tobacco from 1R6F reference cigarettes. Numbers indicate the number of compounds identified in each chemical class. The absence of a compound is indicated by "—".

Chemical Class	Flue-cured	Oriental (Turkish)	Burley	Dark air-cured	1R6F filler
Carboxylic acids	1	1	—	1	1
Furan derivatives	2	4	2	2	3
Pyranones / lactones	4	1	—	—	1
Hydroxy ketones	—	—	—	—	—
Ketones / diketones	—	4	2	—	2
Phenolics	1	2	1	1	1
Monoterpenes	2	—	2	2	1
Norisoprenoids	2	2	1	2	1
Sesquiterpenes	—	1	2	1	1
Sesquiterpenoid alcohols	1	1	1	1	1
Sesquiterpenoid diols	—	—	1	1	—
Diterpenes / diterpenoids	2	3	2	3	2
Bipyridine derivatives	1	1	1	1	1
Nitrogen heterocycles	—	1	—	—	1

These aroma descriptions came from published sources, not from direct sensory testing in this study.

Overall, this study shows that TSP–GC–MS is a useful method for studying natural flavor compounds released from tobacco at temperatures below burning. Using this approach, several additional flavor compounds were identified that may be released in heated tobacco product aerosols. These findings improve understanding of how natural tobacco chemicals influence aroma and flavor under low-temperature (200–325 °C) and provide a basis for future studies linking chemical profiles to aerosol formation and sensory perception.

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Table 3. Estimated natural flavor of research tobaccos and tobacco from 1R6F reference cigarettes.

Sample	Aroma	Taste
Flue-cured	Rich, sweet, and fruity; caramel-like from high pyranones; citrus/pine from monoterpenes and norisoprenoids	Mild acidity; light phenolic character; slightly woody or nutty undertones
Oriental (Turkish)	Complex and layered; smoky-caramel and roasted from furans and ketones; spicy-dry from sesquiterpenes and diterpenes	Astringent and slightly bitter; phenolic and dry aftertaste; lingering spice
Burley	Subtle aroma; low in sweet lactones; earthy and woody from sesquiterpenes; minor smoky hints from furans	Noticeably bitter and dry; less acidic; mild nutty/burnt note from diketones
Dark air-cured	Bold and heavy; roasted and resinous from furans and diterpenoids; earthy-spicy from terpenes	Full-bodied and bitter; phenolic and leathery taste with slight smoky-charred notes
1R6F Filler	Mixed aroma; moderately sweet and woody; pyranones add caramel note; dry herbal from diterpenes	Balanced but slightly bitter; dry and faintly astringent, likely due to phenolics and furans

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ABSTRACT

Artemisia annua (A. annua) herb is renowned for its potent antimalarial and broader biological activities. This study aims to explore the potential of *A. annua* for adding therapeutic compounds to low-temperature heated, tobacco-related products. The volatile compounds were released at four different temperatures (100°C to 325°C) and were analyzed using a Thermal Separation Probe (TSP) coupled with GC-MS. A diverse array of compounds was found, with up to 20 different terpenes identified at certain temperatures. Common terpenes found in dry and fresh tissue included Camphene, β-Sabinene, Eucalyptol, y-Terpinene, Neophytadiene, and Deoxyartemisinin. The potential use of *A. annua* in low-heated tobacco devices for therapeutic advantage is thus considered highly plausible.

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KEYWORDS
Artemisia; thermal separation probe; heated tobacco devices

Introduction

Importance of Species from Genus Artemisia

Artemisia annua (Asteraceae), commonly known as sweet wormwood, is a highly aromatic herb native to China and now naturalized in various temperate and tropical regions worldwide, including Asia, Central and Eastern Europe, India, and North America.[1] With over 500 species within the genus *Artemisia*, *A. annua* has long been valued in traditional Chinese medicine, where the herb has been used for over 2,000 years to treat a range of conditions, including fevers, malaria, inflammation, headaches, and bleeding.[1,2]

A. annua is of particular interest due to its potent antimalarial properties as well as its broader biological activities, including antimicrobial effects. The plant's diverse secondary metabolites contribute significantly to its therapeutic potential.[2] Extensive research has been conducted to identify the specific chemicals responsible for these medicinal properties and to determine the factors influencing their yield and bioactivity.[3–8]

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Studies on *A. annua* volatiles have employed various extraction methods, including steam and hydro distillation, organic solvent extraction, and headspace sampling.[1] These extracts have demonstrated a range of activities such as antiplasmodial, antiviral, antimicrobial, antitumor, anti-inflammatory, and antioxidant effects.[2] The composition of these extracts and their biological activities have been detailed in numerous research publications and reviews.[1,2,9,10] A review highlighted the primary components of these extracts, focusing on monoterpenes and sesquiterpenes.[6] Consistently, three main components – artemisia ketone, 1,8-cineole (eucalyptol), and camphor – have been identified, although their relative abundance varies depending on the plant's geographic origin. However, emissions from low-temperature-heated (e.g. 100°C-325°C) *A. annua* have not been extensively studied.

Artemisia Annua and Herbal Cigarettes

A. annua, known for its aromatic and medicinal properties, is among a variety of herbs that produce fragrant and therapeutically beneficial compounds. Other herbs often used in smoke/combusted herbal cigarettes with similar properties include peppermint (*Mentha piperita*, Lamiaceae), ginseng (*Panax ginseng*, Araliaceae), lavender (*Lavandula angustifolia*, Lamiaceae), thyme (*Thymus vulgaris*, Lamiaceae), and clove (*Eugenia caryophyllata*, Myrtaceae).[11] These plant species have been extensively utilized as food spices, dietary supplements, and herbal medicines. These have also been incorporated into herbal cigarettes. Such products are marketed for purposes including aromatherapy, smoking cessation, and some medical benefits. Detailed information on the content, major active constituents, pharmacological activities, and risks associated with herbal cigarettes are provided in several reviews.[11,12]

Herbal cigarettes are often perceived as a healthier alternative to traditional tobacco cigarettes because they generally lack tobacco and nicotine, the primary harmful substances in conventional cigarettes. They are used as smoking cessation aids, offering a similar to tobacco sensory experience without addictive components. Additionally, some herbal cigarettes are formulated with calming or soothing herbs, contributing to relaxation and aromatherapy.[11] The effectiveness of different herbs in herbal cigarettes varies with the temperature at which their volatiles are released. For example, herbs like catnip, lavender, and lemon balm release their volatiles effectively at temperatures below 150°C, while peppermint, green tea, and yerba mate require temperatures between 150–190°C. Herbs such as valerian, damiana, and chamomile release volatiles at temperatures above 190°C.[13]

Most herbal cigarettes are produced in Asia, where they are increasingly marketed as part of the tobacco harm reduction trend. However,

despite the purported benefits, Asian tobacco companies have yet to provide conclusive evidence supporting these health claims, and there is limited information on the potential harmful effects of herbal cigarettes.[12]

A. annua, though apparently not used in commercial herbal cigarettes thus far, presents intriguing potential due to its significant antimicrobial and anti-inflammatory properties. These characteristics could be advantageous in low-temperature delivery products such as HTP and e-liquids by potentially reducing respiratory infections and inflammation, offering a less harmful alternative to traditional tobacco products.[14] Traditionally, extracts of *A. annua* have been employed to treat respiratory conditions such as bronchitis and asthma, suggesting that incorporating this herb into herbal cigarettes, HTP, and e-liquids could provide soothing effects on the respiratory system.[15] Additionally, the antioxidant-rich nature of *A. annua* may help counteract oxidative stress induced by smoking.[16]

Despite these potential benefits, it is crucial to acknowledge that inhaling any form of smoke can be detrimental to lung health. Therefore, the safety and efficacy of incorporating *A. annua* into herbal cigarettes, HTP and e-liquids require rigorous scientific validation to ensure that they provide a safer alternative to combusted cigarettes.[11]

Justification of Current Research

To date, there is a lack of published information regarding the chemical compounds released during the burning of *A. annua*, as well as the volatiles released at lower temperatures as used in HTP and e-liquid vaping technologies. In the cases of herbal and tobacco cigarettes heating is to combustion (perhaps to 700°C). For herbal teas, volatiles are generally released into water at 100°C. The development of alternatives to traditional smoking products, such as HTP devices and e-liquid vaporizers, represents a crucial step toward combustible cigarettes harm reduction.

This study aims to investigate the volatiles released from *A. annua* when subjected to heat-not-burn temperatures, specifically ranging from 100°C to 325°C. It is sought to explore the potential of incorporating A. *annua* into the tobacco and vaping markets, particularly focusing on its application in HTP products. This investigation included an analysis of the volatiles emitted by *A. annua* under these conditions and evaluated the opportunities to deliver its valuable medicinal and flavor compounds *via* heat-not-burn devices.

Materials and Methods

Plant Material and Sample Collection

For this study seeds from the Swiss variety *A. annua*, known as Apollon were used. The plants were cultivated at the Maine Chance Farm, University of Kentucky's experimental station in Lexington, KY, USA. The seeds were sown on April 27, 2022, in a float system within a greenhouse under ambient light conditions. On June 15, 2022, the seedlings were transplanted into the field using a mechanical transplanter. The plants were harvested at the onset of flowering on September 26, 2022. Immediately following harvest the plants were hung to air-dry in at ambient temperature for several days. After drying, the leaves were ground into 1 mm particles using a Wiley Mill. Fresh leaves were cut to small pieces and analyzed two to ten days post-harvest.

Methods

In herbal HTP and herbal vaping devices, the temperature varies from 100 to 350°C. To study Artemisia volatiles at similar lower temperatures, the Thermal Separation Probe (TSP) application was utilized.

The principle of using the TSP is as follows: TSP is an adapter connected to the inlet of the gas chromatography (GC) column, into which a micro vial with a probe is inserted. TSP utilizes the GC multimode inlet to introduce the sample into the system. The inlet split mode minimizes column and detector overload as well as detector contamination.

The inlet and the GC column have independent temperature programming. Only the compounds vaporized at a specific inlet temperature are carried by the carrier gas into the column and detector. Non-vaporized compounds with high boiling points remain inside the micro vials and can be discarded after each run.[17]

In the current research, the leaf material (5 mg) was placed into an ultra-inert micro vial (Agilent Part No 5190–3187) which was then inserted into a TSP adapter (Agilent Part No: G4382–6300). The TSP was introduced into the Multimode Inlet (MMI). This setup allows for the release of volatile and semi-volatile compounds from the leaf matrix in the TSP inlet before entering the GC column. Only those compounds vaporized at the inlet temperature were carried with the helium gas into the column and detector. This method minimizes cross-contamination between samples (Agilent Technologies; Thermal Separation Probe).[17]

A method, modified from the one described in the application note by Agilent Technologies, Inc., for the analysis of alcohols, was employed.[18] The following instrumentation was utilized in the experiments: Gas Chromatograph: Agilent 8890 GC System, Mass Spectrometer: Agilent

5977B GC MSD, Software: Agilent Mass Hunter (Agilent Technologies) for GC-MS (https://www.agilent.com/en/promotions/masshunter-mass-spec).

Gas Chromatography Conditions: Column: Agilent J&W DB-624 Ultra Inert column (20 m × 180 µm × 1 µm; length, internal diameter, film thickness); Carrier: Helium, 99.9% purity; constant flow of 1.2 mL min^{-1} set at 50°C. Oven temperature program: Initial temperature – 50°C, (held for 1 min); ramp rate – 4°C min^{-1} to 220°C; final temperature – 220°C; total run time – 57.5 minutes. Inlet: Split mode; split ratio – 50:1. Four separate inlet temperatures — 100°C, 200°C, 275°C, and 325°C — were used.

Spectrometry (MSD) conditions were as follows: The ion source temperature was set to 230°C. The interface temperature operated within a range of 250°C to 320°C. The ionization mode used was Electron Ionization (EI), with electron energy of 10 eV.The quadrupole temperature was maintained at150°C. The mass range in scan mode was from 30.00 to 550.00 m/z; scanning rate was 2.8 scans per second. The detector used was an electron multiplier.

Sample Processing

No preliminary sample processing, extraction, purification, or derivatization was required. Plant material, either fresh or dry, was directly inserted into the TSP.

Data Processing

Total Ion Chromatograms (TICs) were obtained in triplicate for each specified temperature. Compound identification was carried out using the NIST Mass Spectral Library, version 17.1, and only compounds having a quality score of ≥ 90% were selected. The retention indices of the identified terpenes were compared with those reported in the literature.[19]

Statistical analysis was performed using R-project for statistical computing.[20] Only the compounds that appeared in at least two out of three replicates were selected and proceeded further. This process was repeated for each of the four temperatures (100°C, 200°C, 275°C, and 325°C). The potential flavor value of the identified compounds was assessed using the relevant resources online.[21,22]

The data were organized into tables showing the identified chemicals at each of the four temperatures (Tables 1 and 2). Additionally, the total volatiles from dried powder and fresh *A. annua* leaves were compared, and their medicinal and flavor potential was shown in separate columns (Table 3).

Note: While “terpenes” and “terpenoids” (or isoprenoids) are often used interchangeably, terpenoids are modified terpenes that contain additional functional groups, typically oxygen-containing (https://en.wikipedia.org/

Table 1. Detailed breakdown of the volatiles emitted from dry *A. annua* powder at four temperatures.

		100℃		200℃		275℃		325℃	
CAS	Library/ID	Average peak area/mg, x 10^6	% of total	Average peak area/mg, x 10^6	% of total	Average peak area/mg, x 10^6	% of total	Average peak area/mg, x 10^6	% of total
000064-19-7	Acetic acid	–	–	–	–	–	–	46.394 ± 15.8	*7.6*
000108–01–0	N, N–Dimethylaminoethanol	–	–	–	–	19.856 ± 8.022	*3.4*	18.163 ± 6.859	*3.0*
000108–38–3	Benzene, 1,3–dimethyl–	–	–	–	–	–	–	2.138 ± 0.346	*0.3*
000629–20–9	1,3,5,7–Cyclooctatetraene	–	–	–	–	–	–	2.720 ± 0.06	*0.4*
000098–00–0	2–Furanmethanol	–	–	–	–	21.541 ± 4.773	*3.7*	25.321 ± 8.135	*4.2*
000079–92–5	Camphene	–	–	1.518 ± 0.748	*0.7*	3.201 ± 0.619	0.6	5.044 ± 2.082	*0.8*
000185–94–4	Bicyclo[2.1.0]pentane	–	–	–	–	–	–	1.756 ± 0.01	*0.3*
003387–41–5	Sabinene	–	–	1.061 ± 1.499	*0.5*	–	–	–	–
000527–84–4	o–Cymene	–	–	3.650 ± 0.953	*1.6*	–	–	–	–
000099–87–6	p–Cymene	–	–	–	–	–	–	7.349 ± 2.021	*1.2*
000470–82–6	Eucalyptol	0.668 ± 0.252	*1.1*	4.639 ± 1.297	*2.0*	4.528 ± 0.416	*0.8*	5.852 ± 1.885	*1.0*
000099–85–4	γ–Terpinene	–	–	–	–	1.059 ± 0.245	*0.2*	1.118 ± 0.26	*0.2*
054151–38–1	1–(1′–Pyrrolidinyl)–2–propanone	–	–	–	–	15.177 ± 3.756	*2.7*	–	–
000765–70–8	Cyclotene	–	–	–	–	–	–	2.423 ± 1.155	*0.4*
017699–16–0	Sabinene hydrate	0.377 ± 0.126	*0.6*	1.499 ± 0.137	*0.7*	–	–	–	–
000108–95–2	Phenol	–	–	–	–	40.478 ± 0.35	*7.1*	10.662 ± 0.853	*1.7*
000090–05–1	Phenol, 2–methoxy–	–	–	–	–	21.027 ± 1.664	*3.7*	28.048 ± 1.291	*4.6*
000473–06–3	Chrisanthenone	2.634 ± 0.644	*4.4*	–	–	–	–	–	–
000473–06–3	β Pinene	–	–	4.486 ± 0.744	*2.0*	–	–	–	–
021835–01–8	Ethyl cyclopentenolone,	–	–	–	–	0.728 ± 0.102	*0.2*	2.107 ± 0.082	*0.3*
000464–49–3	D–Camphor	42.29 ± 7.965	*70.6*	103.146 ± 9.649	*45.1*	112.886 ± 7.703	*19.7*	105.909 ± 15.413	*17.4*
000106–44–5	p–Cresol	–	–	–	–	–	–	2.543 ± 2.268	*0.4*
030460–92–5	Pinocarvone	–	–	0.920 ± 0.085	*0.4*	–	–	–	
000507–70–0	endo–Borneol	+	–	8.853 ± 0.39	*3.9*	10.522 ± 1.635	*1.8*	13.253 ± 1.712	*2.2*
004030–18–6	Pyrrolidine, 1–acetyl–	–	–	4.732 ± 0.14	*2.1*	–	–	14.065 ± 0.821	*2.3*
000099–48–9	Carveol	–	–	2.399 ± 0.691	*1.0*	–	–	–	–
000645–59–0	Benzenepropanenitrile	–	–	–	–	5.361 ± 1.888	*0.9*	–	–
002785–89–9	4–Ethyl guaiacol	–	–	–	–	5.217 ± 0.984	*0.9*	7.899 ± 0.142	*1.3*
003856–25–5	Copaene	1.024 ± 0.988	*1.7*	1.921 ± 0.903	*0.8*	5.099 ± 0.619	*0.9*	6.448 ± 0.95	*1.1*
007786–61–0	2–Methoxy–4–vinylphenol	–	–	3.163 ± 0.429	*1.3*	47.594 ± 8.544	*8.3*	56.863 ± 2.56	*9.3*
033746–71–3	(Z)–Tagetenone	–	–	–	–	0.532 ± 0.131	*0.1*	–	–
000120–72–9	Indole	–	–	–	–	17.846 ± 3.436	*3.1*	33.483 ± 0.759	*5.5*

(*Continued*)

Table 1. (Continued).

		100°C		200°C		275°C		325°C	
CAS	Library/ID	Average peak area/mg, x 10^6	% of total	Average peak area/mg, x 10^6	% of total	Average peak area/mg, x 10^6	% of total	Average peak area/mg, x 10^6	% of total
000091–10–1	Phenol, 2,6–dimethoxy–	–	–	–	–	5.526 ± 1.664	*1.1*	0.788 ± 0.25	*0.1*
000087–44–5	Caryophyllene	4.105 ± 1.282	*6.9*	15.061 ± 2.421	*6.6*	17.237 ± 2.104	*3.0*	20.833 ± 2.741	*3.4*
028973–97–9	cis–β–Farnesene	0.91 ± 0.279	*1.5*	6.123 ± 1.562	*2.7*	7.218 ± 1.785	*1.3*	9.384 ± 4.883	*1.5*
000488–10–8	Jasmone	–	–	1.36 ± 0.01	*0.6*	1.594 ± 0.235	*0.3*	1.52 ± 0.495	*0.3*
1000062–61–9	1,4,7–Cycloundecatriene, 1,5,9,9–tetramethyl–, Z,Z,Z–	–	–	0.742 ± 0.342	*0.3*	–	–	–	–
103827–22–1	Selina–4,11–diene	2.217 ± 0.211	*3.7*	9.275 ± 1.45	*4.1*	11.893 ± 2.369	*2.1*	–	–
000515–17–3	γ–Selinene	–	–	–	–	–	–	14.103 ± 6.428	*2.3*
000123–31–9	Hydroquinone	–	–	–	–	6.674 ± 2.494	*1.2*	5.218 ± 1.91	*0.9*
018252–44–3	β–Copaene	5.696 ± 1.463	*9.5*	–	–	0.272 ± 0.1	*0.1*	2.459 ± 1.042	*0.4*
023986–74–5	Germacrene D	–	–	22.962 ± 2.226	*10.0*	30.186 ± 2.55	*5.3*	27.359 ± 4.93	*4.5*
000483–76–1	(+)–delta–Cadinene	–	–	1.048 ± 0.153	*0.5*	*2.455 ± 0.7*	*0.4*	–	–
016729–01–4	δ–Amorphene	–	–	–	–	–	–	2.367 ± 1.597	*0.4*
027840–40–0	β–Vetivene	–	–	1.293 ± 0.008	*0.6*	–	–	–	–
1000156–12–5	Aromadendrene, dehydro–	–	–	–		1.025 ± 0.501	*0.2*	–	–
000091–64–5	Coumarin	–	–	5.940 ± 2.503	*2.5*	23.995 ± 4.796	*4.2*	26.252 ± 2.017	*4.3*
1000315–88–8	(+)–Coniine	–	–	–	–	–	–	1.098 ± 0.141	*0.2*
006750–60–3	Spathulenol	–	–	8.001 ± 0.548	*3.5*	16.815 ± 1.999	*2.9*	17.924 ± 1.28	*3.0*
036577–33–0	6,9–Guaiadene	–	–	–	–	9.932 ± 0.591	*1.7*	–	–
1000131–71–2	cis–Z–αBisabolene epoxide	–	–	–	–	10.748 ± 0.239	*1.9*	–	–
1000188–66–5	2(1 h) Naphthalenone, 3,5,6,7,8,8a–hexahydro–4,8a–dimethyl–6–(1–methylethenyl)–	–	–	–	–	3.697 ± 0.337	0.6	3.042 ± 0.72	*0.4*
000504–96–1	Neophytadiene	–	–	–	–	57.088 ± 2.265	*10.0*	51.573 ± 6.059	*8.5*
000150–86–7	Phytol	–	–	–	–	3.261 ± 1.563	*0.6*	4.598 ± 2.172	*0.8*
072826–63–2	Deoxyartemisinin	–	–	14.832 ± 3.221	*6.5*	30.411 ± 5.562	*5.4*	21.275 ± 15.385	*3.5*
	SUM	59.921 × 10^6	*100%*	283.971 × 10^6	*100%*	572.669 × 10^6	*100%*	609.353 × 10^6	*100%*
	Terpenes	9		20		20		18	
	Non–Terpenes	0		4		16		19	
	Total compounds	9		24		36		37	

Note: Table 1 includes CAS numbers, the average peak area abundance of each compound per mg of leaf material (in millions), the standard deviation (in millions), and the relative abundance (percentage of total compounds) at each temperature (in italics). The compounds are listed in the order of their appearance in the chromatogram. Terpenes and terpenoids are highlighted in gray color. The absence of chemical is marked with "—".

Table 2. Detailed breakdown of the volatiles emitted from fresh *A. annua* leaves at four temperatures.

		100°C		200°C		275°C		325°C	
CAS	Library/ID,	Average peak area/ mg x 10^6	% of total	Average peak area/ mg x 10^6	% of total	Average peak area/ mg x 10^6	% of total	Average peak area/ mg x 10^6	% of total
000108–38–3	Acetic acid	–	–	–	–	30.517 ±	*11.7*	48.233 ± 1.368	*16.4*
000110–86–1	Pyridine	–	–	–	–	–	–	2.142 ± 0.334	*0.7*
000109–06–8	Pyridine, 2–methyl–	–	–	–	–	–	–	0.886 ± 0.187	*0.3*
000098–01–1	Furfural	–	–	–	–	–	–	4.552 ± 1.022	*1.5*
000930–30–3	2–Cyclopenten–1–one	–	–	–	–	–	–	2.434 ± 0.017	*0.8*
000108–99–6	Pyridine, 3–methyl–	–	–	–	–	–	–	1.554 ± 0.111	*0.5*
000616–43–3	1 h–Pyrrole, 3–methyl–	–	–	–	–	–	–	1.771 ± 0.333	*0.6*
000098–00–0	2–Furanmethanol	–	–	–	–	11.001 ± 5.8	*4.2*	16.427 ± 1.448	*5.6*
000079–92–5	Camphene	8.521 ± 4.693	*10.4*	4.579 ± 1.351	*3.9*	7.182 ± 2.537	*2.8*	5.277 ± 1.418	*1.8*
003387–41–5	β–Sabinene	1.127 ± 0.616	*1.4*	–	–	–	–	–	–
000123–35–3	β–Myrcene	0.296 ± 0.048	*0.4*	–	–	–	–	–	–
000620–02–0	2–Furancarboxaldehyde, 5–methyl–	–	–	–	–	–	–	1.751 ± 0.489	*0.6*
000096–48–0	Butyrolactone	–	–	–	–	–	–	2.504 ± 0.583	*0.8*
003391–86–4	1–Octen–3–ol	0.268 ± 0.088	*0.3*	–	–	–	–	–	–
000099–87–6	p–Cymene	3.335 ± 0.525	*4.1*	0.889 ± 0296	*0.8*	–	–	2.238 ± 0.792	*0.8*
000470–82–6	Eucalyptol	3.985 ± 2.411	*4.8*	4.636 ± 0.994	*4.0*	2.892 ± 0.959	*1.1*	–	–
000099–85–4	γ–Terpinene	0.496 ± 0.375	*0.6*	0.668 ± 0.34	*0.6*	0.521 ± 0.168	*0.2*	–	–
000765–70–8	Cyclotene	–	–	–	–	–	–	6.521 ± 0.849	*2.2*
017699–16–0	cis–Sabinene hydrate	0.464 ± 0.141	*0.6*	2.213 ± 0.666	*1.9*	–	–	–	–
000108–95–2	Phenol	–	–	–	–	16.292 ± 1.118	*6.3*	21.586 ± 2.461	*7.3*
000090–08–1	Phenol, 2–methoxy–	–	–	–	–	3.956 ± 1.185	*1.5*	10.527 ± 2.125	*3.6*
000473–06–3	Chrysanthenon	–	–	2.083 ± 0.64	*1.8*	–	–	–	–
004501–58–0	α–Campholenal	0.254 ± 0.034	*0.3*	–	–	–	–	–	–
018172–67–3	(–)–β–Pinene	–	–	1.529 ± 0.278	*1.3*	3.092 ± 1.669	*1.2*	–	–
000098–01–1	1–Pyrrolidinecarboxaldehyde	–	–	–	–	1.165 ± 0.447	*0.4*	–	–
021835–01–8	Ethyl cyclopentenolone	–	–	–	–	–	–	2.184 ± 0.256	*0.7*
000464–49–3	D–Camphor	43.812 ± 16.37	*53.3*	51.957 ± 3.496	*44.6*	52.48 ± 1.928	*20.2*	–	–
030460–92–5	Pinocarvone	–	–	0.541 ± 0.223	*0.5*	–	–	–	–
000473–67–6	Verbenol	3.305 ± 1.238	*4.0*	–	–	–	–	–	–
000507–70–0	Isoborneol	–	–	–	–	0.486 ± 0.140	*0.2*	2.182 ± 0.547	*0.7*
028564–83–2	4 h–Pyran–4–one, 2,3–dihydro–3,5–dihydroxy–6–methyl	–	–	–	–	–	–	1.11 ± 0.137	*0.4*

(*Continued*)

Table 2. (Continued).

CAS	Library/ID,	100°C		200°C		275°C		325°C	
		Average peak area/ mg x 10^6	% of total	Average peak area/ mg x 10^6	% of total	Average peak area/ mg x 10^6	% of total	Average peak area/ mg x 10^6	% of total
000099–48–9	Carveol	0.286 ± 0.075	*0.3*	0.417 ± 0.203	*0.4*	–	–	–	–
000087–85–4	Benzene, hexamethyl–	–	–	–	–	–	–	1.101 ± 0.183	*0.4*
1000098–14–8	1,4:3,6–Dianhydro–αd–glucopyranose	–	–	–	–	–	–	1.819 ± 0.344	*0.6*
000080–56–8	α–Pinene	–	–	–	–	3.052 ± 1.812	*1.2*	–	–
002785–89–9	4–Ethylguaiacol (phenol–based chemical)	–	–	–	–	–	–	5.336 ± 1.714	*1.8*
000514–95–4	1,5,5–Trimethyl–6–methylene–cyclohexene	–	–	–	–	–	–	7.448 ± 0.388	*2.6*
003242–08–8	γ–Elemene	–	–	–	–	1.594 ± 0.195	*0.6*	–	–
000475–03–6	alpha–Ionone	–	–	–	–	1.37 ± 0.418	*0.5*	0.662 ± 0.179	*0.2*
003856–25–5	Copaene	0.304 ± 0.067	*0.4*	0.99 ± 0.028	*0.9*	1.229 ± 0.199	*0.5*	2.432 ± 0238	*0.8*
007786–61–0	2–Methoxy–4–vinylphenol	–	–	–	–	12.098 ± 2.438	*4.7*	25.472 ± 6.315	*8.6*
000120–72–9	Indole	–	–	–	–	10.241 ± 4.083	*3.9*	8.41 ± 2.59	*2.9*
000091–10–1	Phenol, 2,6–dimethoxy–	–	–	–	–	0.656 ± 0.207	*0.3*	0.437 ± 0.021	*0.1*
000087–44–5	Caryophyllene	2.147 ± 0.732	*2.6*	4.747 ± 0.415	*4.1*	11.887 ± 0.235	*4.6*	11.683 ± 0.65	*4.0*
18794–84–8	β–trans–Farnesene	–	–	3.868 ± 0.995	*3.3*	–	–	–	–
028973–97–9	cis–β–Farnesene	–	–	–	–	3.166 ± 0.945	*1.2*	4.823 ± 3.72	*1.6*
030021–46–6	ε–Muurolene	–	–	–	–	–	–	7.044 ± 0.098	*2.4*
103827–22–1	4a,8–Dimethyl–2–(prop–1–en–2–yl)–1,2,3,4,4a,5,6,7–octahydronaphthalene	5.521 ± 3.219	*6.7*	12.368 ± 9.025	*10.5*	–	–	–	–
000123–31–9	Hydroquinone	–	–	–	–	0.656 ± 0.207	*0.3*	0.321 ± 0.183	*0.2*
000933–67–5	1 h–Indole, 7–methyl–	–	–	–	–	–	–	0.744 ± 0.231	*0.3*
023986–74–5	Germacrene D	4.784 ± 2.861	*5.8*	23.226 ± 3.337	*20.0*	24.902 ± 3.682	*9.6*	38.428 ± 1.764	*13.0*
018252–44–3	β– Copaene	–	–	–	–	5.155 ± 0.968	*2.0*	10.22 ± 0.147	*3.5*
000489–39–4	Aromadendrene	1.128 ± 1.112	*1.4*	1.624 ± 0.07	*1.4*	–	–	–	–
024703–35–3	Bicyclogermacrene	0.667 ± 0.518	*0.8*	–	–	0.533 ± 0.473	*0.2*	–	–
000483–76–1	Cadinene <delta–>	–	–	–	–	–	–	2.381 ± 0.441	*0.8*
000091–64–5	Coumarin	–	–	–	–	8.117 ± 0.978	*3.1*	3.875 ± 1.124	*1.3*
000088–84–6	β–Guaiene	1.473 ± 0.58	*1.8*	–	–	–	–	–	–
000504–96–1	Neophytadiene	–	–	–	–	34.411 ± 1.769	*13.2*	15.911 ± 0.597	*5.4*
072826–63–2	Deoxyartemisinin	–	–	–	–	11.307 ± 5.841	*4.3*	12.283 ± 1.803	*4.2*
	SUM/%	82.173 × 10^6	*100.0*	116.335 × 10^6	*100*	259.958 × 10^6	*100*	294.709 × 10^6	*100.0*
	Terpenes	17		14		16		15	
	Non–Terpenes	2		1		11		25	
	Total compounds	19		15		27		40	

Note: Table 2 includes CAS numbers, the average peak area abundance of each compound per mg of leaf material (in millions), the standard deviation (in millions), and the relative abundance (percentage of total compounds) at each temperature (in italics). The compounds are listed in the order of their appearance in the chromatogram. Terpenes are highlighted in gray color. The absence of chemical is marked with "—".

Table 3. Comparison of terpenes released from fresh and dried *A. annua* and their therapeutic and organoleptic features.

Compound	Dry powder	Fresh leaves	Chemical type	Features		Reference
Camphene	+	+	monoterpene	reduce pain and inflammation, antifungal properties	fragrance additive, mint-like smell	[23]
β-Sabinene	+	+	monoterpene, bicyclic	–	flavor and fragrance	[24]
β-Myrcene	–	+	monoterpene	antioxidant, anti-aging, flammatory, analgesic properties, antibacterial activity	flavoring and aroma agent	[10,25]
p-Cymene	+	–	alkyl benzene, related to monocyclic monoterpenes	–	flavoring, fragrance	[26]
Eucalyptol	+	+	monoterpenoid	controls airway mucus hypersecretion and asthma; antimicrobial	–	[10,23]
γ-Terpinene	+	+	monoterpene	antioxidant, antiproliferative activity	food, flavors, cosmetics, pharmaceuticals, and perfume industries	[10,27]
Sabinene hydrate	+	+	monoterpene	antioxidant, anti-inflammatory, and antimicrobial effects	–	[24]
Chrysanthenone	+	+	terpenoid	an effective anti-inflammatory, a bronchodilator, pain reliever, anxiety-reliever.	–	[27]
α-Campholenal	–	+	monoterpenoid	–	herbal, woody odor	[24]
(-)-β- Pinene	–	+	monoterpene	an effective anti-inflammatory, a bronchodilator; bactericidal activity	vanilla-like odor; used in fragrances and essential oils	[10,26]
β- Pinene	+	–	Monoterpene	anti-inflammatory, anti-microbial, neurological benefits	Fresh, woody, resinous	[10,26]
Camphor	+	+	monoterpenoid	treats colds and congestion; used for cough, pain, and itching.	–	[28]
Pinocarvone	+	+	monoterpenoid	therapeutic properties: calming and sedative; mucolytic	flavor and fragrance, in some essential oils	[21,23]
Verbenol	–	+	monoterpenoid	–	flavoring agent with pine note, food additive, pheromone	[27]
endo-Borneol	+	+	monoterpenoid	improved digestion and blood circulation	distinctively camphor-like aroma; balsamic odor type	[21,24]
Isoborneol	–	+	bicyclic monoterpenoid	antibiotic, anti-fungal, and antiviral properties	flavoring agent; used in perfumery	[29,30]
Carveol	+	+	monoterpenoid	antioxidant	flavoring agent, odor and flavor of spearmint and caraway	[23,27]
γ-Elemene	–	+	sesquiterpene	exhibit antileishmanial activity-	–	[31]
Copaene	+	+	sesquiterpene	anti-inflammatory, healing, antitumor; analgesic, antibacterial, antifungal,	Used in flavor and food Industry.	[23,27]

(*Continued*)

Table 3. (Continued).

Compound	Dry powder	Fresh leaves	Chemical type	Features		Reference
Caryophyllene	+	+	sesquiterpene	anti-inflammatory drug,	musky and spicy fragrance, insect attractant	[27]
cis-β-Farnesene	+	+	sesquiterpene	correlation for anticancer activity	flavoring and fragrance, woody citrus herbal sweet	[21]
β-trans-Farnesene	–	+	sesquiterpene	–	in perfume industry, natural insect repellent	[27]
Jasmone	+	–	terpenoid	antioxidant	jasmine flavor; floral, green, jasmine odor	[32]
1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	+	–	sesquiterpene	–	natural product, insect repellant	
γ-Selinene	+	–	sesquiterpene	–	in essential oils	[27]
(Z)-tagetenone	+	–	diterpenoid	Extractible from Tagetes species exhibit antioxidant, anti-inflammatory, and enzyme inhibitory properties.	–	[27]
β-Copaene	+	+	sesquiterpene	antioxidant properties	used as a food additive and flavorant	[21]
Aromandendrene	–	+	sesquiterpene	anti-bacterial, -inflammatory, -cancer, especially when combined with β-caryophyllene	–	[29]
Bicyclogermacrene	–	+	sesquiterpene	eliminates mosquito larvae	odor type: green, woody weedy	[21,33]
Germacrene D	+	+	sesquiterpene	potential cytotoxic effect on HA cells; antibacterial and antifungal activities	herbal, basil- like aroma, slightly floral	[23]
γ (iso)-Cadinene	+	+	sesquiterpene	–	fragrance, cosmetic, and pharmaceutical industries	[23]
beta-Vetivenene	+	–	sesquiterpene	–	natural substance and extractive	
β-Guaiene	–	+	sesquiterpene	–	odor and flavor – woody	[21]
6,9-Guaiadene	+	–	sesquiterpene	–	natural product, pheromone	
Spathulenol	+	–	3-cyclic sesquiterpenoid	restrains the growth of lymphocytes	earthy herbal fruity odor	[21]
cis-Z-.alpha.-Bisabolene epoxide	+	–	sesquiterpenoid	–	natural product	
Neophytadiene	+	+	diterpene	an anti-inflammatory agent, an antimicrobial agent	–	[27]
Phytol	+	–	diterpenoid	antioxidant, anti-inflammatory, anti-schistosomiasis and antibacterial activities.	mild light floral balsam green jasmine odor	[26]
Deoxyartemisinin	+	+	sesquiterpenoid	anti-inflammatory and antiulcer activities	–	[34]

Note: The presence or absence of chemical is marked with "+" and "-." The compounds that did not exhibit potential valuable properties were not referenced.

wiki/Terpenoid). For simplicity, the term "terpenes" was used throughout this study. The nature of the compounds were detailed (Table 3).

Results

Volatile Composition of Dry Artemisia Leaf Powder

The volatile profile of dry *A. annua* material was analyzed using the same conditions as for fresh leaves, across four temperatures: 100°C, 200°C, 275°C, and 325°C. Each sample was analyzed in triplicate, and the detailed chemical profiles were presented (Table 1).

At 100°C, nine terpenes were identified, representing 100% of the total compounds. D-Camphor was the predominant compound, constituting 70.6% of the total, followed by β-Copaene (9.5%) and Caryophyllene (6.9%). The total peak abundance was 59.921×10^6.

At 200°C, 24 compounds were detected, with a total peak abundance of 283.971×10^6. Among these, 20 were terpenes, representing 83.3% of the total compounds. D-Camphor remained the most abundant (45.1% of all compounds), followed by Germacrene D (10%) and Caryophyllene (6.9%). Other terpenes were present in concentrations of less than 4.1% each.

At 275°C, the number of terpenes remained the same as at 200°C, but the number of non-terpene compounds increased to 16, making up 44% of the total compounds. The total peak abundance doubled compared to 200°C, reaching 572.669×10^6. D-Camphor was still the major terpene (19.7%), followed by Neophytadiene (10%), Deoxyartemisinin (5.4%), and Germacrene D (5.3%). Non-terpene compounds with notable peak abundance included Phenol (7.1%), 2-Methoxy-4-vinylphenol (8.6%), and Coumarin (4.3%).

At 325°C, 37 compounds were identified, including 18 terpenes (49% of total compounds). The overall peak abundance was 609.353×106. D-Camphor continued to be the most abundant terpene (17.4%), followed by Neophytadiene (8.5%), Germacrene D (4.5%), Deoxyartemisinin (3.5%), and Caryophyllene (3.4%). Non-terpenes with notable abundance included Acetate (7.6%), 2-Methoxy-4-vinylphenol (9.3%), and Indole (5.5%).

Several terpenes were detected at all temperatures, including Eucalyptol, D-Camphor, Copaene, Caryophyllene, and *cis*-β-Farnesene. D-Camphor consistently had the highest peak area across all temperatures. However, its relative abundance decreased at higher temperatures due to the increasing presence of non-terpene compounds. A similar trend was observed with Caryophyllene, which also showed reduced relative abundance at higher temperatures.

Non-terpene compounds began to appear at 200°C and their presence increased significantly with temperature increase. At 275°C, non-terpenes

comprised 44% of the total compounds, with 16 non-terpenes detected. This proportion elevated to 51% at 325°C, with 19 non-terpenes identified (Table 1).

At 275°C, the major non-terpene compound was 2-Methoxy-4-vinylphenol (8.3%), followed by Phenol (7.1%) and Coumarin (4.3%). At 325°C, 2-Methoxy-4-vinylphenol increased to 9.3%, while Acetate (7.6%) and Indole (5.5%) became more prominent. The origin of these non-terpenes varies: some are products of carbohydrate degradation (e.g. 2-Furanmethanol, Ethyl cyclopentenolone), while others are natural compounds or remain unidentified. For example, Indole may originate from the decomposition of tryptophan, whereas 2-Methoxy-4-vinylphenol, despite its high abundance, is not a known degradation product in Artemisia, but is a natural product found in other plants. Coumarin, on the other hand, is a natural compound present in Artemisia.[9]

Volatile Composition of Fresh Artemisia Leaves

The list of volatiles emitted from fresh *A. annua* leaves at four different temperatures was listed (Table 2). The table includes the average peak area of each compound expressed in millions ($x10^6$), per mg of leaf material, the standard deviation, and the relative abundance (percentage of total compounds) at each temperature. CAS numbers are included for reference, and compounds are listed in the order of their appearance in the chromatogram. Terpenes and terpenoids are highlighted in gray color.

At 100°C, a total of 17 terpenes were identified, comprising 89.5% of the total released compounds with a peak area abundance of 82.173×10^6. The most abundant compound was D-Camphor, accounting for 53.3% of the total, followed by Camphene (10.4%) and Germacrene D (5.8%). Other terpenes were present in less than 5% each.

At 200°C, 14 terpenes were detected, constituting 93.3% of the total compounds. The peak area increased to 116.335×10^6. D-Camphor remained the predominant compound (44.7%), followed by Germacrene D (20%). The remaining terpenes were present in concentrations of less than 4% each.

Raising the temperature to 275°C resulted in a significant increase in the number of compounds, with a total peak area of ~ 260×10^6. At this temperature, 16 terpenes (59.3% of the total) and 11 additional volatile compounds were identified. The latter are degradation products, derivatives, or compounds requiring higher temperatures to volatilize. The major terpene was again D-Camphor (20.2%), followed by Neophytadiene (13.2%) and Germacrene D (9.6%). New terpenes such as α-Pinene, γ-Elemene, cis-β-Farnesene, β-Copaene, Neophytadiene, and Deoxyartemisinin were detected for the first time at 275°C, and they were present in lower concentrations (4.3%, 1.2%, and 2%, respectively).

At 325°C, the highest number of compounds (37) was identified, including 12 terpenes and 25 non-terpene compounds. The total peak area was 294.709×10^6. Terpenes represented only 32.4% of the total compounds at this temperature. The major terpene identified was Germacrene D (13%), followed by Neophytadiene (5.4%). Notably, D-Camphor, which was predominant at the lower temperatures, was absent at 325°C, and a new terpene, ε-Muurolene, was detected. Non-terpene compounds doubled the number found at 275°C. At 325°C, non-terpene constituents were present in higher relative abundance than terpenes. Some of the non-terpene compounds included Acetic Acid (16.4% of total), 2-Furanmethanol (5.6%), Phenol (7.3%), and 2-Methoxy-4-vinylphenol (8.6%).

Several terpenes were consistently detected across all temperatures: Camphene, Copaene, Caryophyllene, and Germacrene D. The peak area of Camphene varied slightly across the four temperatures, while Caryophyllene showed a double increase in abundance at 275°C and 325°C compared to the lower temperatures. Germacrene D's relative abundance increased substantially (eight-fold) from 4.8×10^6 at 100°C to 38.4×10^6 at 325°C. In contrast, D-Camphor's abundance remained stable regardless of temperature. The organoleptic properties and potential medical benefits of these terpenes were detailed (Table 3).

Additional non-terpene compounds were detected as the inlet temperature increased, with their number rising from one or two at lower temperatures to 11 at 275°C and 25 at 325°C.At 275°C, the predominant non-terpene compounds were Acetic Acid (11.7% of total), Phenol (6.3%), and 2-Methoxy-4-vinylphenol (4.7%). At 325°C, Acetic Acid remained the major non-terpene compound (16.4%), with the highest abundance among both terpenoids and non-terpenoids, followed by 2-Methoxy-4-vinylphenol (8.6%) and Phenol (7.3%). At this temperature, non-terpenes comprised 68% of the total compounds.

Comparison of Volatiles in Dry Vs. Fresh A. Annua

Terpenes

Dry tissue released 29 terpenes, while the fresh tissue released 28 terpenes. Of these, 19 terpenes were common to both tissues, and 20 were unique to either the dry or fresh material. Overall, 39 terpenes were identified across both types of tissue at all temperatures. For dry material, the peak area of D-Camphor was twice as high compared to fresh material and was detectable even at 325°C, a temperature at which it was absent from the fresh leaves. Neophytadiene and Deoxyartemisinin were also more prominent in the dry material. Neophytadiene was detected only at 275°C and 325°C in both dry and fresh leaves. Copaene showed at least a 2.5-fold increase at 275°C and 325°C in both types of material.

Compounds with potential medicinal properties included: Camphene, β-Myrcene, Eucalyptol, Sabinene hydrate, Chrysanthenone, β-Pinene, Camphor, Copaene, Caryophyllene, Cis-β-Farnesene, Aromandendrene, and Deoxyartemisinin. Deoxyartemisinin, while lacking significant anti-malarial activity, has demonstrated anti-inflammatory and antiulcer properties.[35] Fragrant and flavor compounds included: β-Sabinene, p-Cymene, γ-Terpinene, Verbenol, Carveol, iso-Borneol, Cis-β-Farnesene, Jasmone, γ (iso)-Cadinene, and Spathulenol. Some terpenes, such as iso-Borneol, Cis-β-Farnesene, Copaene, and Germacrene D possess multifunctional properties, including therapeutic benefits, organoleptic benefits, and antibacterial properties (Table 3).

Non-Terpenes

In the dry material, 21 non-terpenoids were recorded, compared to 19 in fresh material. Only 10 of these non-terpenes were common to both types of material. The non-terpenes included a mix of natural substances, potential toxins, and flavor compounds (Table 4).

Discussion

The thermal separation method employed to *A. annua* enabled the detection of a broad spectrum of terpenes, including monoterpenes, sesquiterpenes, and diterpenes. The most abundant terpene was D-Camphor, which remained dominant across different temperatures, underscoring its stability and importance in the volatile profile of both dry and fresh *A. annua* leaves.

The variety of *A. annua* used in this study, Apollon, contains glandular trichomes on the abaxial leaf surface (Figure 1). Previous research has established that leaves with glandular trichomes primarily produce monoterpenes, whereas glandless leaves are more inclined to produce sesquiterpenes.[38] In this study, a wide array of monoterpenes/terpenoids, sesquiterpenes/terpenoids, and diterpenes were detected, highlighting the diversity of the volatile profile of the Apollon variety. The use of thermal separation method rather than extraction method allowed for the detection of all terpene types, though sesquiterpene lactones (i.e. artemisinin), which are thermally unstable, were not observed.[39] In the current study of the Swiss Apollon variety of *A. annua*, grown in Kentucky, artemisia ketone and artemisia alcohol were not detected. Artemisia ketone is a compound traditionally associated with artemisinin-rich cultivars of *A. annua*.[40] Instead, the analysis revealed a predominance of camphor. These findings suggest that the Swiss Apollon variety grown on the soil of Kentucky belongs to the camphor chemotype, differing from the Swiss *A. annua* profiles reported in other studies, where camphor was not identified as a major compound.[5,7] This discrepancy underscores the importance of

Table 4. Comparison of non-terpenes released from fresh and dried *A. annua* and their therapeutic and organoleptic features.

Compound	Dry powder	Fresh leaves	Chemical type	Features		References
Butanal, 3-methyl-	–	+	aldehyde derivative of butane	–	flavor type: fruity	[21]
Acetic acid	–	+	organic acid	to treat external otitis	acidic odor, sour flavor	[21]
Pyridine	–	+	heterocyclic organic compound	–	toxic	
N,N-Dimethylaminoethanol	+	–	organic compound	taken orally as a nootropic- improve cognitive functions	–	[26]
Benzene, 1,3-dimethyl-	+	–	aromatic hydrocarbon	–	chemical	
Furfural	–	+	organic compound, aldehyde	–	flavoring agent	[26]
2-Furanmethanol	+	+	furan derivative	–	odor – bready; flavor-burnt, caramellic	[21]
Bicyclo [2.1.0]pentane	+	–	organic, bicyclic compound	–	chemical	
2-Furancarboxaldehyde,5-methyl-	–	+	a member of furans and an aldehyde	–	a spicy and sweet roasted aroma with nutty caramel aspects	[21]
Butyrolactone	–	+	4-carbon lactone	–	tobacco flavor, slightly caramelly, nutty 22	[21]
1-Octen-3-ol	–	+	unsaturated alcohol	–	mushroom smell, natural product; attracts biting insects	[27]
o-Cymene	+	–	aromatic hydrocarbon	–	natural product; neurotoxin	
1-(1′–Pyrrolidinyl)-2-propanone	+	–	n-alkyl pyrrolidine	–	natural product	
Cyclotene	+	+	organic compound	–	food improvement, flavoring agent	[27]
Phenol	+	+	organic compound	relieves pain and irritation in mouth and sore throat	–	[36]
Phenol, 2-methoxy- (o-guaiacol)	+	+	phenol derivative	expectorant, antiseptic, and local anesthetic	spicy flavor and odor	[26]
Ethyl cyclopentenolone	+	+	cyclic ketone	–	flavor and fragrance agent	[21]
p-Cresol	+	–	phenol derivative	–	chemical, toxic	
Pyrrolidine, 1-acetyl-	+	–	pyridine alkaloid	–	chemical	
Benzenepropanenitrile	+	–	a nitrile and a member of benzenes	–	natural product	
4-ethyl guaiacol	+	–	a phenolic compound	–	smoky and clove-like profile with woody and sweet vanilla nuances	[21]
2-Methoxy-4-vinylphenol	+	+	class of phenols, called guaiacols	strong antibacterial properties	spicy flavor and odor, flavoring agent	[21]

(*Continued*)

Table 4. (Continued).

Compound	Dry powder	Fresh leaves	Chemical type	Features		References
Indole	+	+	a type of phytochemicals	-	natural product; used in perfumery	[26]
Phenol, 2,6-dimethoxy-	+	+	phenol derivative	–	the single most important flavor chemical in smoke flavors	[21]
4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4 ,4a,5,6,7-octahydronaphthalene	+	+	naphthalene	–	chemical	
4 h-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	–	+	Oxygenated heterocyclic compound	anti-inflammatory and antioxidant properties	–	[37]
Hydroquinone	–	+	benzenediol	skin bleaching agent, toxic	–	
Coumarin	+	+	benzo-alpha pyrone	–	natural product; toxic	
(+)-Coniine	+	–	alkaloid	–	natural alkaloid; toxic	
2(1 h) Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl -6-(1-methylethenyl)-	+	–	polycyclic aromatic	–	chemical	

Note: The presence or absence of chemical is marked with "+" and "-." The compounds that did not exhibit potential valuable properties were not referenced.

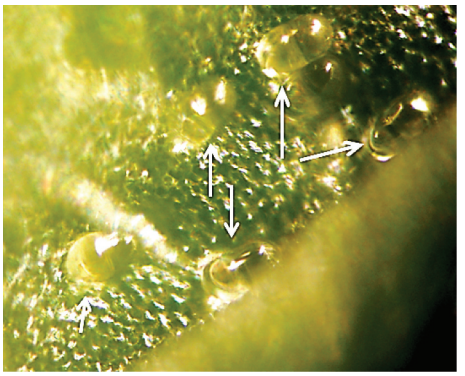

Figure 1. Light microscopy of glandular trichomes of *A. annua* var. Apollon on the abaxial side of the leaf. Note: Picture was taken with Olympus DP71 microscope, bright field at 200× magnification. Illumination was provided from the top with Fiberlight A3200 source (DOLAN-JENNIFER Industries, Inc.). White arrows show the clear and transparent subcuticular sack of several glandular trichomes.

phytogeographic and genetic variability in Artemisia chemotypes and highlights the unique chemical profile of the Swiss Apollon variety.[5]

A comparison between dry and fresh *A. annua* leaves revealed that both tissue types emitted a similar range of terpenes, when heated at low temperatures, many of which are known for their medicinal, flavor, and fragrance properties. Volatiles common to both types of tissue included Camphene, β-Sabinene, Eucalyptol, γ-Terpinene, Sabinene hydrate, Chrysanthenone, Camphor, Pinocarvone, Borneol, Carveol, Copaene, β -Copaene, Caryophyllene, cis-β-Farnesene, Germacrene D, and γ-(iso)-Cadinene, Neophytadiene, and Deoxyartemisinin. Some terpenes were present individually in dry or fresh leaves.

These findings suggest that *A. annua* could be an effective component in heated tobacco devices, herbal cigarettes, or e-liquids, offering both recreational and medicinal benefits. The terpenes from dry powder (32) and fresh leaf (30), could enhance the therapeutic value and aromatic qualities of products. Additionally, the ability to release these beneficial compounds at temperatures as low as 100°C provides an opportunity to optimize the efficiency and safety of such devices by reducing the need for higher operating temperatures, which can lead to the formation of harmful by-products.

The non-terpenes predominantly appeared at higher temperatures possibly due to thermal degradation and transformation processes, or due to required higher temperature for release from the leaf matrix. Compounds such as 2-Furanmethanol, Butyrolactone, Furfural, Phenol, 4 h-Pyran-4-one, and 2,3-Dihydro-3,5-Dihydroxy-6-methyl likely result from the breakdown of sugars.[41] Other non-terpenes, such as 2-Cyclopenten-1-one, 1 h-Pyrrole, Cyclotene, Ethyl Cyclopentenolone, and 2-Methoxy-4-vinylphenol, are recognized as natural products.[21] Pyridine, may arise

from the degradation of pyridine alkaloids, while the Benzene hexamethyl, comes from unclear source. The appearance of these non-terpene compounds at higher temperatures suggests that heating processes can lead to the formation of new chemical species through thermal interactions. While some of these non-terpenes may contribute to the plant's aroma and flavor, others could potentially form harmful substances, indicating that precise temperature regulation is crucial in minimizing the risk of degradation products.

The analysis of dry *A. annua* further confirmed that non-terpenes become more prominent at higher temperatures. These findings underscore the importance of balancing temperature to preserve beneficial compounds while minimizing the creation of unwanted by-products.

The current investigation into the volatiles of *A. annua*, generated by heating leaves at temperatures ranging from 100°C to 325°C for less than 30 seconds, uncovered up to 40 different compounds (terpenes and non-terpenes) at certain temperatures, many of which are recognized for their strong antibacterial and antifungal properties, further supporting the medicinal potential of *A. annua* in both traditional and modern applications.

Ultimately, the comparison of volatiles released from fresh and dry artemisia leaves points to substantial similarities between the two, though some differences were observed in the concentration of specific compounds. In particular dry *A. annua* appears well-suited for use in such devices, while fresh *A. annua* is more appropriate for oil extraction for medicinal and fragrance purposes, or as an additive in cigarette sticks and e-cigarettes.

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Disclosure Statement

No potential conflict of interest was reported by the author(s).

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Author Contribution

AMK: Project Administration, Conceptualization, Methodology, Data Analysis, Writing – Original Draft, Writing – Review & Editing.
VK: Experiments' execution, Data Processing.
GW: Overall consultation, Discussion, Writing – Review & Editing.
JK: Artemisia Growth in Field and Green House, Tissue preparation, Writing – Some Methodology.
All authors read and approved the final manuscript.

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FISCAL YEAR 2025 – 2026

FINANCIAL REPORT

April 1, 2026 – June 30, 2026

QUARTERLY REPORT

TOBACCO RESEARCH INCOME INCOME COMPARISON						
Fiscal Years	2020-2021	2021-2022	2022-2023	2023-2024	2024-2025	2025-2026
July	$ 136,565.92	$ 102,816.87	$ 113,853.04	$ -	$ 97,579.97	$ 84,421.42
August	$ 11,873.82	$ 148,863.59	$ 121,485.75	$ 235,814.07	$ 113,878.38	$ 94,787.78
September	$ 261,157.23	$ 138,395.19	$ 143,503.64	$ 116,834.55	$ 112,212.24	$ 109,920.98
1st QUARTER	**$ 409,596.97**	**$ 390,075.65**	**$ 378,842.43**	**$ 352,648.62**	**$ 323,670.59**	**$ 289,130.18**
October	$ 141,682.93	$ 138,913.78	$ 131,512.77	$ 84,290.07	$ 86,565.34	$ 96,740.93
November	$ 135,157.14	$ 101,844.54	$ 101,050.68	$ 132,736.05	$ 88,478.89	$ 104,074.91
December	$ 159,616.92	$ 138,232.14	$ 113,515.64	$ 81,648.61	$ 90,136.26	$ 97,484.98
2nd QUARTER	**$ 436,456.99**	**$ 378,990.46**	**$ 346,079.09**	**$ 298,674.73**	**$ 265,180.49**	$ 298,300.82
January	$ 93,056.96	$ 116,044.01	$ 111,657.62	$ 101,501.91	$ 78,472.89	$ 80,225.85
February	$ 125,797.09	$ 89,271.71	$ 78,955.86	$ 77,922.09	$ 125,701.11	$ 73,327.03
March	$ 143,903.75	$ 140,521.53	$ 119,175.49	$ 105,636.69	$ 45,037.80	$ 95,603.89
3rd QUARTER	**$ 362,757.80**	**$ 345,837.25**	**$ 309,788.97**	**$ 285,060.69**	**$ 249,211.80**	$ 249,156.77
April	$ 144,970.47	$ 127,449.97	$ 79,639.90	$ 119,161.48	$ 94,449.22	$ 117,024.15
May	$ 100,238.76	$ 148,769.94	$ 120,890.24	$ 88,889.28	$ 80,887.20	$ 85,184.03
June	$ 211,130.06	$ 121,204.33	$ 149,991.92	$ 154,407.96	$ 103,211.51	$ 98,631.88
4th QUARTER	**$ 456,339.29**	**$ 397,424.24**	**$ 350,522.06**	**$ 362,458.72**	**$ 278,547.93**	$ 300,840.06
TOTAL INCOME	**$ 1,665,151.05**	**$ 1,512,327.60**	**$ 1,385,232.55**	**$ 1,298,842.76**	**$ 1,116,610.81**	**$ 1,137,427.83**

Funds Center	Funds Center Name	Category	Original Budget	Annual Budget	Prior Month Balance	Current Month Actual	YTD Actual	YTD Encumbrances	Available Budget
1235410080	KTRDC HOLDING ACCOUNT	Revenue	($1,723,000.00)	($1,723,000.00)	($1,038,795.95)	($98,631.88)	($1,137,427.83)		($585,572.17)
1235410080	Result	Total	($1,723,000.00)	($1,723,000.00)	($1,038,795.95)	($98,631.88)	($1,137,427.83)		($585,572.17)
1235410090	KENTUCKY TOBACCO RESEARCH BOARD	Operating Expenses		$1,000.00	$97.18		$97.18		$902.82
1235410090	Result	Total		$1,000.00	$97.18		$97.18		$902.82
1235410100	KTRDC ADMINISTRATION	Salaries	$1,723,000.00	$260,000.00	$152,437.74	$11,473.84	$163,911.58		$96,088.42
1235410100	KTRDC ADMINISTRATION	Benefits			$61,205.08	$4,323.84	$65,528.92		($65,528.92)
1235410100	KTRDC ADMINISTRATION	Operating Expenses			$8,287.40	$1,772.59	$10,059.99		($10,059.99)
1235410100	KTRDC ADMINISTRATION	Recharges			$58.66	$3.95	$62.61		($62.61)
1235410100	Result	Total	$1,723,000.00	$260,000.00	$221,988.88	$17,574.22	$239,563.10		$20,436.90
1235410110	KTRDC PERSONNEL	Salaries			$353,095.41	$30,156.67	$383,252.08		($383,252.08)
1235410110	KTRDC PERSONNEL	Benefits			$105,383.34	$9,655.86	$115,039.20		($115,039.20)
1235410110	KTRDC PERSONNEL	Operating Expenses		$1,000,000.00	$9,618.93	($397.11)	$9,221.82		$990,778.18
1235410110	KTRDC PERSONNEL	Recharges			$42,415.63	$3,505.05	$45,920.68		($45,920.68)
1235410110	KTRDC PERSONNEL	Transfers			$63.68		$63.68		($63.68)
1235410110	Result	Total		$1,000,000.00	$510,576.99	$42,920.47	$553,497.46		$446,502.54
1235410120	KTRDC PUBLICATIONS AND TRAVEL	Operating Expenses		$25,000.00	$11,813.80	$7,478.96	$19,292.76		$5,707.24
1235410120	KTRDC PUBLICATIONS AND TRAVEL	Recharges			$909.50	$6.74	$916.24		($916.24)
1235410120	Result	Total		$25,000.00	$12,723.30	$7,485.70	$20,209.00		$4,791.00
1235410130	KTRDC BUILDING MAINTENANCE	Operating Expenses		$50,000.00	$22,123.77	$1,134.80	$23,258.57		$26,741.43
1235410130	KTRDC BUILDING MAINTENANCE	Recharges			$5,978.83	$1,225.50	$7,204.33		($7,204.33)
1235410130	Result	Total		$50,000.00	$28,102.60	$2,360.30	$30,462.90		$19,537.10
1235410180	KTRDC SHOP	Operating Expenses		$2,000.00	$1,128.70		$1,128.70		$871.30
1235410180	KTRDC SHOP	Recharges			$8.47		$8.47		($8.47)
1235410180	Result	Total		$2,000.00	$1,137.17		$1,137.17		$862.83
1235410240	KTRDC LABORATORY EQUIPMENT	Operating Expenses		$40,000.00	$20,337.34	$2,497.75	$22,835.09		$17,164.91
1235410240	Result	Total		$40,000.00	$20,337.34	$2,497.75	$22,835.09		$17,164.91
1235410250	KTRDC UNALLOCATED RESERVE FOR RESEARCH	Operating Expenses		$90,000.00					$90,000.00
1235410250	Result	Total		$90,000.00					$90,000.00
1235410280	KTRDC GENERAL LABORATORY	Operating Expenses		$50,000.00	$15,070.77	$1,266.28	$16,337.05		$33,662.95
1235410280	KTRDC GENERAL LABORATORY	Recharges			$1,503.78	$6.42	$1,510.20		($1,510.20)
1235410280	Result	Total		$50,000.00	$16,574.55	$1,272.70	$17,847.25		$32,152.75

Funds Center	Funds Center Name	Category	Original Budget	Annual Budget	Prior Month Balance	Current Month Actual	YTD Actual	YTD Encumbrances	Available Budget
1235411040	KTRDC DISCRETIONARY	Operating Expenses		$20,000.00	$4,226.21	$590.08	$4,816.29		$15,183.71
1235411040	KTRDC DISCRETIONARY	Recharges			$47.83	$6.31	$54.14		($54.14)
1235411040	Result	Total		$20,000.00	$4,274.04	$596.39	$4,870.43		$15,129.57
1235411310	KTRDC OUTREACH & COMMUNICATIONS	Operating Expenses		$20,000.00					$20,000.00
1235411310	Result	Total		$20,000.00			$0.00		$20,000.00
1235411340	GENETIC MANIPULATION OF TOBACCO TO	Salaries			$20,924.75		$20,924.75		($20,924.75)
1235411340	GENETIC MANIPULATION OF TOBACCO TO	Benefits			$4,089.12		$4,089.12		($4,089.12)
1235411340	GENETIC MANIPULATION OF TOBACCO TO	Operating Expenses		$30,000.00					$30,000.00
1235411340	GENETIC MANIPULATION OF TOBACCO TO	Recharges			$4,985.00		$4,985.00		($4,985.00)
1235411340	Result	Total		$30,000.00	$29,998.87		$29,998.87		$1.13
1235411360	PLANT BIOTECH METABOLIC	Operating Expenses		$30,000.00	$28,795.62	$4,852.57	$33,648.19	$23.37	($3,671.56)
1235411360	PLANT BIOTECH METABOLIC	Recharges			$294.18	$31.57	$325.75		($325.75)
1235411360	Result	Total		$30,000.00	$29,089.80	$4,884.14	$33,973.94	$23.37	($3,997.31)
1235411370	KTRDC PLANT BIOTECH - MOLECULAR	Operating Expenses		$30,000.00	$23,901.95	$459.56	$24,361.51		$5,638.49
1235411370	KTRDC PLANT BIOTECH - MOLECULAR	Recharges			$5,137.30	$21.01	$5,158.31		($5,158.31)
1235411370	Result	Total		$30,000.00	$29,039.25	$480.57	$29,519.82		$480.18
1235411380	MOLECULAR GENETICS	Operating Expenses		$30,000.00	$8,388.79	$3,932.60	$12,321.39		$17,678.61
1235411380	MOLECULAR GENETICS	Recharges			$2,253.63	$31.38	$2,285.01		($2,285.01)
1235411380	Result	Total		$30,000.00	$10,642.42	$3,963.98	$14,606.40		$15,393.60
1235411410	KTRDC GREENHOUSE	Operating Expenses		$15,000.00	$12,133.50	$1,352.81	$13,486.31		$1,513.69
1235411410	KTRDC GREENHOUSE	Recharges			$3,515.57	$80.90	$3,596.47		($3,596.47)
1235411410	Result	Total		$15,000.00	$15,649.07	$1,433.71	$17,082.78		($2,082.78)
1235411570	TOBACCO MOLECULAR FARMING AGRONOMICS	Recharges			$0.00		$0.00		$0.00
1235411570	Result	Total			$0.00		$0.00		$0.00
1235412360	FLAVONOID - SMALLE	Salaries			$15,739.06	$1,391.72	$17,130.78		($17,130.78)
1235412360	FLAVONOID - SMALLE	Benefits			$7,538.13	$694.61	$8,232.74		($8,232.74)
1235412360	FLAVONOID - SMALLE	Operating Expenses		$30,000.00	$231.65	$5.25	$236.90		$29,763.10
1235412360	FLAVONOID - SMALLE	Recharges			$256.23	$36.51	$292.74		($292.74)
1235412360	Result	Total		$30,000.00	$23,765.07	$2,128.09	$25,893.16		$4,106.84

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