Chapter 6
Artemisia annua and *Artemisia afra* Essential Oils and Their Therapeutic Potential

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Abbreviations

ACT Artemisinin combination therapy
EO Essential oil

6.1 Introduction

For millennia, *Artemisia annua* L. was used by the Chinese to treat fever, which was often thought to be malaria (Hsu 2006; Tu 2011). The sesquiterpene lactone, artemisinin, is considered the main antimalarial phytochemical in *A. annua* (Fig. 6.1). However, many constituents of the plant's essential oils (EOs) including 1,8-cineole (eucalyptol), limonene, myrcene, α- and β-pinenes, and nerolidol also are known to be antimalarial as isolated chemicals, albeit with much less effective inhibitory concentrations (IC_{50} values) than artemisinin (see reviews by Weathers et al. 2014, 2017). There is evidence, however, suggesting that at least in some cases, the EO fraction per se is more potent than its individual constituents (Radulović et al. 2013). *A. afra* has also been used by native Africans to treat malaria (Liu et al. 2009; Watt and Breyer-Brandwijk 1962). While considerable information is known about the breadth of the medicinal properties of *A. annua* to treat not only malaria as well as other diseases, *A. afra* has only recently attracted more attention for its healing properties (Patil et al. 2011). Although their composition is somewhat different, constituents of the EOs of each species seem to play a role in the therapeutic efficacy of both plant species. Here, we summarize what is currently known about the EO fraction of these two important medicinal plant species and how the phytochemicals therein may affect therapeutic outcomes.

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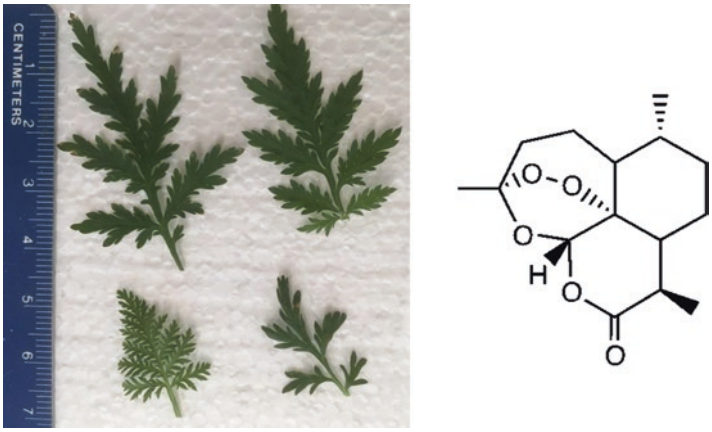

Fig. 6.1 Representative leaves of the same developmental age from three cultivars of *Artemisia annua* and one *A. afra* along with structure of artemisinin. Clockwise from top left: *A. annua* SAM, #15, GLS, *A. afra*

6.2 Essential Oil Content of *A. annua*

6.2.1 Cultivar Content Differences

One of the major difficulties in studying EOs from *A. annua* is the inconsistency of the oil contents. From cultivar to cultivar, the major constituents of the oil can change dramatically. Even within cultivars, factors including geographic location, growth conditions, method of propagation, and stage of development at harvest can change the contents of the oil. For example, our lab performed a phytochemical analysis of *A. annua* essential oil sourced from the United States or China. The resulting peak areas from the GC-MS analysis can be seen in Table 6.1 (Desrosiers and Weathers 2017). As expected, both oils contained mostly monoterpenes; however, the oil sourced in the United States contained almost 31% thujone, a compound not detectable in the Chinese-sourced oil. Although thujone is characteristically found in *A. afra* and *A. absinthium*, *A. annua* is consistently reported to be thujone-free, which aids in its validation as a generally recognized as safe (GRAS) plant (Duke 2001). The presence of thujone, therefore, suggests the US-sourced EO was adulterated with oil from other *Artemisia* species. The US-sourced oil contained about 30% camphor, while the Chinese oil only contained about 14% of the same. Such dramatic differences in EO composition even within the same plant species (see below) make studying the oil and establishing reliable scientific conclusions difficult. Comparisons between labs are nearly impossible, and this raises questions about therapeutic uses of the oil if a consistent product cannot be produced on a large scale. This also highlights the dangers of using unregulated EO products to treat medical conditions. An EO of undefined chemical makeup cannot be trusted to consistently treat any medical condition and may even have undesirable health effects.

Table 6.1 Relative abundance of some phytochemicals identified by GC-MS in *A. annua* essential oil from US and Chinese sources (Desrosiers and Weathers 2017)

Phytochemical	US EO source % of total peak area	Chinese EO source % of total peak area
1,8-Cineole (eucalyptol)	16.5	27.4
α-Pinene	0.3	17.5
β-Pinene	2.5	4.7
Borneol	3.7	1.8
Camphene	13.2	5.5
Camphor	30.3	14.1
Carene	Nd	2.2
Caryophyllene	1.0	6.2
Caryophyllene oxide	0.9	Nd
Copaene	Nd	1.1
Humulene	Nd	5.5
Limonene	Nd	5.2
Myrcene	0.1	Nd
Phellandrene	Nd	8.5
Santolina triene	0.1	0.2
Stigmasterol	0.1	Nd
Terpineol	0.2	Nd
Thujone	30.9	Nd

EO essential oil, *Nd* not detected

6.2.2 *Changes Throughout Plant Development*

As *A. annua* growth shifts from the vegetative to reproductive stage, EO components change, and maturity of the leaf is also a factor (Bagchi et al. 2003; Rana et al. 2013; Towler and Weathers 2015; Yang et al. 2012). For example, 1,8-cineole content decreases for the *A. annua* SAM clonal line in vegetative leaves versus leaves from plants in the budding (reproductive) stage. Vegetative leaves also have no detectable α-pinene, but it is present in floral budding plants, and camphor is nearly threefold higher in young leaves of the shoot apical meristem region versus mature leaves (Towler and Weathers 2015). However, as previously noted, different cultivars have varying EO profiles, along with unpredictable responses to the morphological changes associated with plant maturation. Each cultivar would need to be studied in a particular environment and growth stage in order to obtain a reasonable description of its "typical" EO content. Such developmental variations in EOs would also be expected for *A. afra*.

6.2.3 Artemisia afra

Why *A. afra*? This species is native to southern Africa and has been used as a tea infusion by indigenous peoples to treat fevers, especially associated with malaria (Watt and Breyer-Brandwijk 1962). Many other medicinal properties are ascribed to the plant; these are well summarized in two relatively recent reviews (Liu et al. 2009; Patil et al. 2011). Recently, tea infusions of *A. afra* and *A. annua* performed faster than praziquantel in treating schistosomiasis in a human clinical trial (Munyangi et al. 2018). The oil also shows antimicrobial activity against some bacteria and yeast species. Unfortunately, most of the studies express antibiotic activity through a series of oil dilutions or by zones of inhibition; only a few studies provide IC_{50} values, statistical analysis of minimum inhibitory concentration (MIC), or minimum inhibitory percentage (MIP). In a recent malaria clinical trial, a tea infusion of *A. afra* performed similarly to *A. annua,* and both were better than artemisinin combination therapy (ACT) in their therapeutic efficacy and reduction of gametocyte carriage (Munyangi et al. 2019). Interestingly, *A. afra* used in that study only had trace amounts of artemisinin.

Over 130 volatile chemicals constituting *A. afra* EO were identified and documented in a review by Liu et al. (Liu et al. 2009). The most common volatiles in *A. afra* include artemisyl acetate, 1,8-cineole, α- and β-thujone, artemisia ketone, α-copaene, camphor, santolina alcohol, borneol, and camphene (Liu et al. 2009). As observed for *A. annua*, these EO phytochemicals change in quantity and quality with plant part, among cultivars, throughout development, with cultivation, and with processing method. For example, surveying some of the volatile constituents of dried leaf samples of three cultivars analyzed in our lab, camphor can vary by threefold and thujone can be present or absent (Table 6.2). Drying methods also substantially alter volatile components from 0.18% to 1.88% from fresh to dried material (Asekun et al. 2007). Considering that fresh material has about ten times the water of dried material, these percent values are not particularly different. However, drying did change the relative composition of individual phytochemicals within the oil. As an example, artemisia ketone was present in fresh material but absent in air- and sun-dried material (Asekun et al. 2007). When comparing the effects of microwave-drying against air-, sun-, and oven-dried material, the monoterpenes, 1,8-cineole and β-thujone (but not α-thujone) decreased, while several other compounds increased, particularly *trans*-caryophyllene (Ashafa and Pitso 2014).

6.2.4 Caveats About Extraction and Analysis

Methods of producing EOs include steam distillation, solvent extraction, CO_2 extraction, maceration, enfleurage, cold-press extraction, and water distillation. Factors such as temperature, pressure, and processing time all affect the quality and

Table 6.2 Example of essential oil variation in *A. afra* cultivars

	Phytochemical (mg g DW^{-1})		
Cultivar	PAR	SEN	WPI
1,8-Cineole (eucalyptol)	0.47	0.27	2.68
a-Pinene	Nd	Nd	0.02
β-Neoclovene #	0.51	0.13	4.32
β-Pinene	Nd	Nd	0.02
Borneol #	0.67	0.07	0.55
Camphor	3.26	0.72	2.90
Caryophyllene	Nd	Nd	Nd
Caryophyllene oxide	Nd	Nd	Nd
Spathulenol #	0.12	Nd	0.28
Thujone	0.86	Nd	Nd
Other important phytochemicals			
Artemisinin	Nd	0.05	Nd
Total flavonoids +	3.74	3.03	7.95

Methylene chloride extract assayed directly by GC-MS. Each of the three cultivars were dried leaves from plants grown in Senegal (SEN), Paris (PAR), or at Worcester Polytechnic Institute (WPI), with the latter sourced from Companion Plants, Athens, OH, USA. Each plant cultivar had an $n = 6$; Nd = not detected; all identified using NIST library; #, expressed as camphor equivalents; +, expressed as quercetin equivalents; all others quantified with authentic standards

composition of the resulting EO. In terms of identification and quantification of the phytochemicals in a given EO product, it can be very difficult to make comparisons among content claims. Most notably, it is important to be aware that, particularly for GC-MS analysis, the ion current generated by a compound depends on its characteristics and is not a true measure of quantification (Tzenkova et al. 2010). EOs are often described by listing identifiable components and their respective peak areas, however, this is an estimation. In addition, compound identification is not infallible. Reference standards are needed for accurate quantification, and it is nearly impossible or prohibitively expensive to procure them for every compound present within an EO mixture. Of note, the source of the *A. annua* EO from China shown in Table 6.1 claimed that it had a high content of artemisia ketone; however, it was undetectable.

6.3 Therapeutic Efficacy of *A. annua* Essential Oils

6.3.1 Diversity of Therapeutic Efficacy

Artemisinin from *A. annua* has been widely studied for its antimalarial activity, and several derivatives have been developed as ACTs and are in use to combat malaria worldwide. However, the EO produced by *Artemisia* species also has wide-ranging antimicrobial properties. Many have pondered the evolutionary benefits of producing

EO, as well as artemisinin, to the *A. annua* plant. Some have speculated these compounds acted as antimicrobials and insecticides to deter herbivorous insects and pathogenic microbes. There is indeed evidence for these hypotheses, as several studies have shown *A. annua* EO to have activity against common bacterial and fungal strains, with examples shown in Table 6.3.

While the EO of *A. annua* has shown some promise in vitro, there are few studies in vivo and there are still several questions surrounding the use of EOs as a therapeutic. For example, the proper way to deliver EOs is not clear. For certain external infections, topical application may suffice, but in vivo studies and clinical trials would have to be performed to establish efficacy, dosage, and safety. Furthermore, it is unclear whether or not it is economically feasible to produce EOs on a large enough scale to be used as a therapeutic. Large-scale production of EOs requires steam distillation of large amounts of plant material to produce a small amount of oil, and for this reason, it may simply be too expensive to rely on EOs as antimicrobials.

Table 6.3 Antimicrobial activity of *A. annua* essential oil

Microbial species	Activity	Reference
Candida albicans *Saccharomyces cerevisiae* *Enterococcus hirae*	Growth inhibition	Juteau et al. (2002)
Listeria innocua	No activity	Viuda-Martos et al. (2010)
Staphylococcus aureus *Bacillus cereus* *Enterococcus faecalis* *Pseudomonas aeruginosa* *Escherichia coli*	Growth inhibition and bactericidal	Massiha et al. (2013)
Staphylococcus aureus *Escherichia coli* *Bacillus subtilis* *Bacillus thuringiensis*	Growth inhibition	Li et al. (2011)
Staphylococcus aureus *Escherichia coli* *Saccharomyces cerevisiae* *Candida albicans*	Growth inhibition	Verdian-Rizi et al. (2008)
Pseudomonas aeruginosa	No activity	
Bacillus cereus *Staphylococcus aureus* *Sarcina lutea* *Escherichia coli* *Klebsiella pneumoniae* *Salmonella enteritidis* *Shigella* sp. *Candida albicans* *Aspergillus fumigatus*	Growth inhibition	Radulović et al. (2013)

6.3.2 Toxicity: Humans

Although any pure EO can be toxic, consumption of *A. annua* EOs as part of dried leaf material or a tea infusion is nontoxic; the plant is GRAS, and tea infusions have been consumed for millennia. *A. afra* may contain thujone in its EO, which is considered toxic and, therefore, regulated. Thujones act on the central nervous system as antagonists of γ-aminobutyric acid-A ($GABA_A$) (Höld et al. 2000) and 5-hydroxytryptamine (5-HT_3, or serotonin) (Deiml et al. 2004), but the toxicity of *A. afra* is debatable. Interestingly, the EU restricts thujone to 0.5 mg kg^{-1} in food prepared with *Artemisia* species, but in those made with sage, which also contains the monoterpene, the limit is 25 mg kg^{-1}. There are also similar types of restrictions for foods and beverages in the USA and Canada, again with exceptions for use of the herb, sage. In rats, the per os LD50 is 500 mg kg^{-1}, and thus, naturally occurring levels of thujone in *A. afra* are too low from orally consumed plant material either as powdered dried leaves or as tea infusions to be considered toxic (Table 6.2). Additionally, only a small fraction of the thujone present in plant material is extracted into water compared to an ethanolic formulation (Tegtmeier and Harnischfeger 1994).

6.3.3 Process Caveats: Losses with Drying, Storage, Powdering, Tableting

Fresh *A. annua* plant material has a different EO profile versus dried, processed leaves, mainly due to the volatile nature of the monoterpene components. After fresh leaves were dried, sieved, and powdered, camphor and 1,8-cineole content decreased. Only camphor remained detectable after powdered leaves were processed into tablets via mechanical compression (Table 6.4; adapted from Weathers and Towler 2014). Powdering dried leaf material with a blade mill generates only slight heat when performed in short pulses. However, operation of the machinery required to form the tablets can cause an increase in processing temperature, which can

Table 6.4 Selected compounds in *A. annua* and effect of drying, granulation, and tablet formation

Compound	Fresh leaves[a] mg g^{-1} DW^{-1}	Dried and sieved mg g^{-1} DW^{-1}	Powdered mg g^{-1} DW^{-1}	Tablets mg g^{-1} DW^{-1}
1,8-cineole	0.30	0.03	0.03	Nd
Camphor	3.57	2.10	1.67	0.19
Artemisinin	11.38	15.90	17.31	17.18
Total flavonoids #	1.55	2.78	5.05	10.97

[a]DW calculated using DW/FW ratio of 0.25. Methylene chloride extract assayed by GC-MS, except total flavonoids assayed by colorimetric aluminum chloride assay. Each condition had $n \geq 6$; nd = not detected; #, expressed as quercetin equivalents; all others quantified with authentic standards and identified using NIST library

account for the loss of monoterpenes. We simulated different processing methods of *A. annua* leaves on a small scale by comparing a commercial coffee mill that has a flat blade (Kitchen Aide) to a mortar and pestle, representing cutting and crushing, respectively. Compared to powdering via cutting, analysis of five components of the *A. annua* EO powdered using mortar and pestle showed a percent loss of 40, 100, 100, 15, and 0 for camphor, α-pinene, eucalyptol, caryophyllene, and phytol, respectively. Artemisinin and total flavonoid content responded differently; drying and processing had a neutral or positive effect on the measured concentrations (Table 6.4). Processing variations also affected *A. afra* EOs (Ashafa and Pitso 2014).

We also monitored the stability of the dried leaf material in storage by tracking the total flavonoid content and several EO monoterpenes in dried *A. annua* leaves kept in a sealed plastic bag at room temperature in dim light. While the amount of artemisinin and total flavonoids remained relatively stable for over a year, the monoterpene fraction declined over time. After 2 months, we observed that camphor and 1,8-cineole content dropped by 25%; after 2 years, camphor content decreased by over 60% and 1,8-cineole by nearly 90%.

This information collectively emphasizes the importance of selecting the appropriate processing equipment and performing assays on the final consumer-ready product and not just the starting material. The EO profile is particularly susceptible to processing changes.

6.4 Bioavailability

Beyond its direct bioactivity against microbes, the EO of *A. annua* has effects on the bioavailability of the main drug of interest in *A. annua*, artemisinin, at least indirectly increasing the therapeutic efficacy of dried leaf treatment. The oral bioavailability of any drug is influenced by several factors as illustrated in Fig. 6.2. An orally delivered drug is first subject to low pH and digestive enzymes in the stomach. It is then subject to more enzymatic activity, bile, and more neutral pH in the small intestine. Most absorption occurs in the intestine where solubility of the drug has significant effects on how well the drug is absorbed. The drug also is subjected to limited first-pass metabolism as it is transported across the intestine and then travels

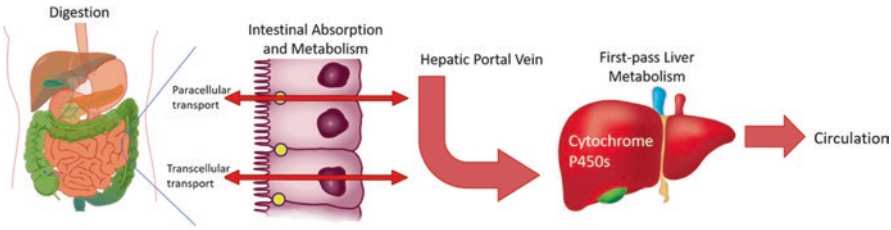

Fig. 6.2 Schematic of the steps orally delivered drugs take to reach systemic circulation

through the hepatic portal vein to the liver where it is then subjected to significant metabolism before reaching systemic circulation. *A. annua* EO may have effects at each stage in this process, and those effects are discussed here. For a more comprehensive review of oral drug bioavailability, please see Song et al. (2004).

6.4.1 Solubility Effects

A. annua EOs are largely found in the glandular trichomes where they co-localize with artemisinin, allowing it to stay in solution. Traditionally, artemisinin is known to have low bioavailability due largely in part to its low aqueous solubility. In its pure form, the drug does not readily solubilize in aqueous environments like the stomach or intestine, leading to very low absorption into the blood. As a result, pure artemisinin treatment is only marginally effective against malaria because most of the drug does not reach systemic circulation where it is required to act on the *Plasmodium* parasites. Consequently, the derivatives of artemisinin currently used in ACTs were developed to have increased aqueous solubility. Interestingly, when artemisinin is delivered as plant material, artemisinin solubility is about fourfold higher in simulated intestinal fluid (Desrosiers and Weathers 2016). EO from the plant is largely responsible for this effect. When *A. annua* EO was combined with pure artemisinin and subjected to simulated digestion experiments, the solubility in simulated intestinal fluid increased 2.5-fold (Desrosiers and Weathers 2016). This effect on solubility partially explains why artemisinin delivered as dried *A. annua* leaves was >40× more bioavailable than pure drug in mice (Weathers et al. 2011). Other antimalarial phytochemicals in *A. annua* also have low aqueous solubility; for example, the flavonoids, which are reported to synergistically enhance antimalarial efficacy of artemisinin (Liu et al. 1992; Suberu et al. 2013). Bioavailability of these phytochemicals may also be increased. Thus, by increasing solubility of artemisinin and other phytochemicals, the EO in the plant also increases the bioavailability of these compounds, further enhancing the antiplasmodial efficacy of artemisinin delivered as powdered dried plant leaves.

6.4.2 Intestinal Transport

Besides altering artemisinin solubility, the EO of *A. annua* altered the intestinal permeability of artemisinin. Artemisinin is transported across the intestine through simple diffusion (Augustijns et al. 1996), and our group has shown its intestinal permeability is increased by 37% when delivered as digested *A. annua* leaves (Desrosiers and Weathers 2017). In studies using the Caco-2 cell model of the intestinal epithelium, *A. annua* EO that had been subjected to simulated digestion decreased the intestinal permeability of artemisinin. However, when EO from *A. annua* and dried leaves of an *A. annua* mutant lacking glandular trichomes and

artemisinin were digested together with pure artemisinin using the simulated system, the permeability of artemisinin was unchanged compared to pure artemisinin controls (Desrosiers and Weathers 2017). These data suggested that *A. annua* EO decreased permeability on its own and that this decrease was nullified by the bulk of the plant matrix.

6.4.3 Potential Effects on the Liver

The full story of how *A. annua* EOs affect artemisinin bioavailability is not complete without understanding how EOs affect metabolism in the liver, where artemisinin is known to undergo significant first-pass metabolism. In this scenario, artemisinin is metabolized into four metabolites: deoxyartemisinin, deoxydihydroartemisinin, 9,10-dihydrodeoxyartemisinin, and crystal 7 (Lee and Hufford 1990). None has any antimalarial activity mainly due to the loss of the endoperoxide bridge responsible for the drug's potent activity against *Plasmodium*. In addition to solubility concerns, the high first-pass metabolism of artemisinin is another reason for the development of the semi-synthetic artemisinin derivatives currently used in ACTs. These semi-synthetic derivatives are metabolized in the liver into dihydroartemisinin, which retains the endoperoxide bridge and potent antiplasmodial activity (Lee and Hufford 1990; Navaratnam et al. 2000).

Several compounds found in the EO of *A. annua* may, however, modulate first-pass liver metabolism of artemisinin in a way that allows more of the drug to reach systemic circulation. For example, camphor, one of the components found in both *A. annua* and *A. afra* EOs, inhibits CYP2B6, the main enzyme responsible for artemisinin metabolism (Seo et al. 2008; Svensson and Ashton 1999). By inhibiting CYP2B6, camphor present in the oil would allow more artemisinin to bypass metabolism in the liver and reach systemic circulation. Borneol, limonene, and cineol also inhibited CYP2B6. More studies are needed to determine if other components in *A. annua* EO inhibit CYP2B6 as well as CYP3A4, which is also partially involved in artemisinin metabolism.

6.5 Repellency and Insecticidal Activity

Besides having a myriad of antimicrobial activities, *A. annua* EOs have been reported to have various insecticidal and repellant qualities, including activities against stored product beetles, codling moths (*Cydia pomonella*), blowflies (*Calliphora vomitoria*), *E. paenulata,* and *S. eridania* (Table 6.5).

As with their use as antimicrobials, questions remain about the economic feasibility of these compounds as insecticides. Nevertheless, EOs may offer a more environmentally friendly alternative to some common synthetic insecticides. Many have also postulated that *A. annua* EO may function as an effective mosquito repellant;

Table 6.5 Repellant and insecticidal activity of *A. annua* essential oil and essential oil components on various insect species

A. annua component	Species	Activity	Source
A. annua alcoholic extract	*Cydia pomonella*	Repellant	Durden et al. (2011)
Artemisinin			
1,8-Cineole			
A. annua EO	*Calliphora vomitoria*	Insecticidal	Bedini et al. (2017)
A. annua EO	*Tribolium castaneum*	Repellant and insecticidal	Tripathi et al. (2000)
	Callosobruchus maculatus	Insecticidal	

however, to our knowledge, no reliable studies have been conducted to date to validate this claim. Many of the EOs found in *A. annua* are also in *A. afra* and would be expected to provide similar responses.

6.6 Conclusions

While EOs per se are not recommended as direct therapeutic agents, their inclusion in an herbal or other medicinal preparation may have profound effects on therapeutic outcomes. Evidence shows that EOs from *A. annua* and *A. afra* may not only have direct therapeutic effects against various ailments including infectious diseases but also enhance the bioavailability of more potent phytochemical drugs, e.g., artemisinin. The mechanism of action of EO effects include improved solubility of otherwise poorly soluble compounds and possible inhibition of liver metabolism by cytochrome P450s. Overall, this information enhances our understanding of the role of EOs in therapeutic medicinal applications.

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