Cytotoxicity of Ethanolic Extracts of *Artemisia annua* to Molt-4 Human Leukemia Cells

Authors Narendra P. Singh[1], Jorge F. S. Ferreira[2], Ji Sun Park[1], Henry C. Lai[1]

Affiliations
[1] Department of Bioengineering, University of Washington, Seattle, WA, USA
[2] Appalachian Farming Systems Research Center, USDA-ARS, Beaver, WV, USA

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- *Artemisia annua*
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Abstract

Although dihydroartemisinin (DHA) and other artemisinin derivatives have selective toxicity towards cancer cells, *Artemisia annua* (*A. annua*) extracts containing artemisinin have not been evaluated for their anticancer potential. Our main goal was to assess the anticancer effect of ethanolic leaf extracts of *A. annua* from Brazilian and Chinese origins (with DHA as a comparison) on normal and cancer cells. Leukocytes and leukemia (Molt-4) cells were counted at 0, 24, 48, and 72 hr after treatment with extracts having artemisinin concentrations of 0, 3.48, 6.96, and 13.92 µg/mL. Also, we assessed the antioxidant capacity of these extracts using the oxygen radical absorbance capacity (ORAC) test. Both extracts had high antioxidant capacity and toxicity towards Molt-4 cells. DHA was significantly more potent ($p < 0.05$) in killing Molt-4 cells than Brazilian extract at 48 and 72 hr and Chinese extract at 72 hr. In Molt-4 cells, LD_{50} values for Brazilian and Chinese extracts were comparable at all time points and not significantly different from DHA at 24 hr. In leukocytes, DHA, Chinese extract, and Brazilian extract had LD_{50} values of 760.42, 13.79, and 28.23 µg/mL of artemisinin, respectively, indicating a better safety index for the Brazilian extract compared to that of the Chinese extract at 24 hr. However, at 48 and 72 hr, the toxicity in leukocytes for any of the treatment groups was not significantly different. These experiments suggest that these extracts may have potential application in cancer treatment.

Introduction

Artemisia annua Linnaeus, family Asteracea (*A. annua*), has been used for at least 1600 years in traditional Chinese herbal medicine to treat symptoms associated with malaria. *Herba Artemisiae annuae* (leaves + stems) was cited in the *Classified Materia Medica* as a food supplement related to longevity [1], a characteristic probably associated with its antiparasitic and antioxidant properties. *A. annua* is still the sole commercial source of artemisinin.

Dihydroartemisinin (DHA), a semisynthetic derivative of artemisinin, kills cancer cells *in vitro* [2, 3] and *in vivo* [4–6], leading to most anticancer research currently performed with DHA [7–9]. No evaluation of the potential anticancer properties of *A. annua* crude extracts has been published. Although ethanolic, aqueous, and other plant extracts are commercially available [10], the concentration of their bioactive metabolites and the origin of their plant source are undisclosed.

Chloroform crude extracts of *A. annua* are rich in methoxylated flavonoids that synergized artemisinin antiparasitic activity [11]. However, the nonpolar (lipophilic) nature of these extract makes their testing *in vitro* challenging. On the other hand, hydrophilic (polar) extracts are easy to dissolve in aqueous medium. In addition, the lyophilized ethanolic (70% EtOH) leaf extract used in this study had hydrophilic oxygen radical absorbance capacity (ORAC) of 2535 µmoles TE/g DW [12]. Considering that antioxidant phenolics (including flavonoids such as quercetin and casticin) present in *A. annua* leaves have anticancer activity of their own [13], we hypothesize that ethanolic leaf extracts of *A. annua* have similar anticancer effects as DHA. In addition, *Artemisia* flavonoids may synergize with artemisinin, increasing its biological activity [14].

To our knowledge, this is the first study in which *A. annua* crude ethanolic extracts have been assessed for their anticancer activity. Our goal was to evaluate the anticancer activity of two ethanolic *A. annua* extracts (from Chinese and Brazil-

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Correspondence
Dr. Narendra P. Singh
Department of Bioengineering
University of Washington
Box 355061
Seattle, WA 98195-5061
USA
Phone: + 1 206 68 5 20 60
Fax: + 1 206 68 5 39 25
narendra@u.washington.edu

ian origins), using DHA as a comparison (in concentrations similar to those that have been previously used) against Molt-4, a cancer cell line derived from human lymphoblastoid leukemia cells.

Materials and Methods

Plant material and growing conditions

Artemisia annua of both Brazilian (University of Campinas, Brazil, cultivar 3 M) and Chinese (Chongqing Holley Pharma Company, Chongqing, China) origins were cultivated in soil type Gilpin silt loam (fine-loamy, mixed, mesic Typic Hapludult) in Beaver, West Virginia, USA, at 37°45′ N, 80°59′ W (945 m altitude). Seedlings were greenhouse-grown under a 16-hr photoperiod and field transplanted when 20 cm high in June. Plants had a 1 plant · m^{-2} density and received 45 kg N, 20 kg P, and 37 kg K per hectare at planting. Both Brazilian and Chinese cultivars were test-cultivated in West Virginia for three years (2005-2007) and produced peak artemisinin between late August and the first week of September [15], with artemisinin concentrations ranging from 0.7–1.5%. Both cultivars were identified by one of the authors (JFSF), an expert on the species, and were confirmed as *Artemisia annua* L. (Asteraceae). In addition, although three species out of approximately 400 have been reported to produce artemisinin, only selected *A. annua*, such as the cultivars used here, produce artemisinin at levels high enough to be of commercial interest.

Materials

Ethanol, acetonitrile, and petroleum ether were all HPLC-grade (Fisher Scientific). Artemisinin (Sigma-Aldrich, 98% pure), dihydroartemisinic acid (92.2% pure), and artemisinic acid (> 90% pure) both from Amyris, as well as deoxyartemisinin (96.2% purity, kind gift of Dr. Mitchell Avery) were used as standards to identify and quantify compounds from plant extracts. For the ORAC analysis, fluorescein sodium salt, AAPH [2,2′-azobis (2-methylpropionamidine) dihydrochloride], Trolox (a vitamin E water soluble analog, over 98% pure), K_2HPO_4 dibasic, and $NaH_2PO_4 \cdot H_2O$ monobasic were purchased from Fisher Scientific or Sigma-Aldrich. The ORAC assay was performed according to a preestablished method [16].

For the *in vitro* cell culture experiment, unless mentioned otherwise, all chemicals were purchased from Sigma-Aldrich.

Preparation of ethanolic extracts and HPLC-UV analysis

Leaves of Brazilian and Chinese cultivars were dried in a forced-air oven at 45 °C for 48 hr and then ground in a cyclone grinder to 2-mm size particles and kept at −15 °C until extraction. Ground dry leaves (25.0 g) of each cultivar were extracted using 150 mL of 70% aqueous ethanol (70 : 30 ethanol: water) at 60 °C with stirring for 2 hr. Extracts were sonicated for 30 min and filtered through Whatman #2 filters. Extraction and sonication were repeated, extracts combined, rotary evaporated and sonicated in pure ethanol to remove all extract from the evaporation flask. No trace of ethanol remained after lyophilization. Extracts were concentrated under nitrogen and freeze-dried. This resulted in 8.37 g (33.5% yield) of green crystals for the Brazilian *Artemisia* and 6.44 g (25.74% yield) for the Chinese *Artemisia*. Crystals were powdered using mortar and pestle before *in vitro* studies and antioxidant analysis.

To test if 70% EtOH extracts the major *A. annua* sesquiterpenes as commercial nonpolar (hexane or petroleum ether) extraction, another Brazilian clone (3 M-39), which produces 1.5% artemisinin, was extracted and analyzed (HPLC-UV) by a validated procedure [17]. Ethanolic extracts were prepared by adding 10 mL of acetonitrile to 100 mg of each lyophilized extract. These solutions were sonicated for 30 min, filtered and analyzed. The chromatograms (**Fig. 1**) are for comparing phytochemical profiles between hexane extracts and lyophilized ethanolic extracts, but not for concentration comparison due to biomass differences generated by those two extraction methods.

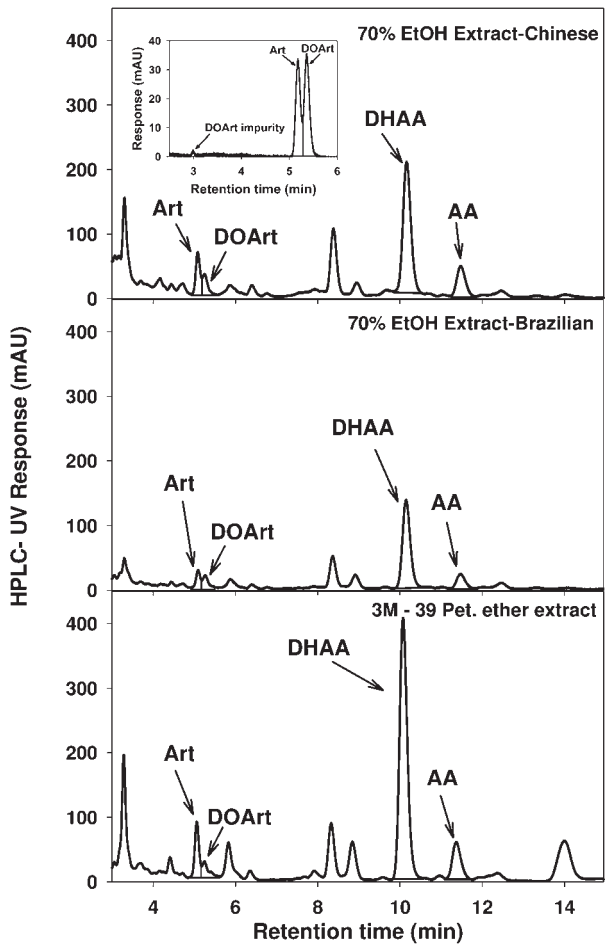

Fig. 1 HPLC-UV chromatograms of crude ethanolic and petroleum ether extracts of *A. annua* (top and bottom). Standards of artemisinin (Art) at 0.25 mg/mL (area = 503.09 mAU*s) and standards of deoxyartemisinin (DOArt) at 0.25 mg/mL (area = 331 mAU*s), 10 µL injection, are shown in the top chromatogram. Ethanolic extracts were from 100 mg samples of each extract dissolved in 10 mL acetonitrile (MeCN), while petroleum ether extracts (for comparison) were generated from refluxing a 500 mg dry leaf sample, drying, and reconstituting in 20 mL of MeCN. Art = artemisinin (r. t. = 5.2 min), DOArt = deoxyartemisinin (r. t. = 5.36 min), DHAA = dihydroartemisinic acid (r. t. = 10.2 min), and AA = artemisinic acid (r. t. = 11.3 min). Ethanolic extracts have all typical component peaks as petroleum ether extract of Brazilian 3 M-39, except peak at r. t. = 14 min.

Oxygen radical absorbance capacity (ORAC) assay

ORAC assay for hydrophilic components (flavonoids and phenolic acids) was performed for both Brazilian and Chinese ethanolic extracts as follows: 100 mg of each lyophilized extract was mixed with 10 mL of acetone : water : acetic acid (70 : 29.5 : 0.5). Solutions were vortexed (K-550-G; VWR Scientific) for 30 sec and

sonicated (FS60H; Fisher Scientific) for 10 min at 37 °C. Solutions were vortexed for 15 sec and centrifuged (Sorval Evolution RC; Thermofisher) at 3000 rpm for 7 min. The supernatant from each centrifuge tube was transferred to a 25-mL volumetric flask. Extraction, sonication, and centrifugation steps were repeated, supernatants combined, and the final volume was brought to 25 mL using the acetone : water : acetic acid solution described above. Extracts were diluted 120 times with phosphate buffer, and 40 µL was transferred to a 48-well Multiwell™ polypropylene plate (Becton Dickinson Labware). To each well, 400 µL of fluorescein and 40 µL of AAPH in phosphate buffer were dispensed automatically. Plates were read in a fluorescence reader (FLUOstar Optima; BMG Labtech) set at 485 nm excitation and 520 nm emission wavelengths. Samples were reacted with AAPH (an oxidizer) and fluorescein at 37 °C and read in 45 cycles, at 3.35-min intervals for 150 min. Antioxidant activity of the extracts was evaluated by their neutralization of the oxidizer AAPH, thus preventing fluorescein oxidation. Antioxidant capacity was calculated by differences between the areas under the curve (AUC) generated by the samples, blank, and standard curves (using AAPH as an oxidizer and Trolox as the antioxidant). Calculations were done by the MARS software (version 1.10, Build 1.10.1.28; BMG Labtech), and results were expressed in µmoles of Trolox equivalents per gram of sample dry weight (µmoles TE/g DW), according to a published procedure [16].

Molt-4 cell culture treatment and counting

Cells used in these experiments were purchased from ATCC (American Type Culture Collection) and were validated to be Molt-4, a human lymphoblastoid leukemia cell line. These cells were grown in 100% humidity at 37 °C in 5% CO_2, in RPMI-1640 medium (Life Technologies) with 10% fetal bovine serum (Hyclone). The cell density was kept around 1.8×10^5 cell/mL to start with. Two hr after adding culture medium to the cells (1 : 1), they were treated with 1 mg/mL human holotransferrin, and aliquots of 1 mL were incubated at 37 °C for 1 hr before addition of diluted plant extracts or DHA.

A. annua ethanolic extracts and DHA were dissolved in 100% DMSO. Molt-4 cell cultures were treated with DHA at 0.88, 1.7, and 3.5 µg/mL or with ethanolic *Artemisia* extracts with artemisinin equivalents of 3.48, 6.96, and 13.92 µg/mL. Final DMSO concentration in all cultures including controls was 1%. As an index of cell growth, Molt-4 cells were counted immediately before (0 hr) and at 24, 48, and 72 hr after treatment using a Reichert light microscope and a hemocytometer as previously described [2].

Leukocyte cell culture treatment and counting

Frozen human leukocytes were purchased from Lonza Walkersville Inc. They were thawed and suspended in complete RPMI-1640 medium and divided into aliquots of 1 mL. The cell density was kept around 1.7×10^5 cell/mL to start with. These samples were subjected to holotransferrin and DHA/extract treatments as described for Molt-4 cells. At 0, 24, 48, and 72 hr of drug treatment, after thoroughly suspending cells, 10 µL of leukocyte samples were mixed well with 10 µL of 10 µg/mL of acridine orange in PBS, and 10 µL of this cell suspension was loaded in a hemocytometer chamber. Total cells were counted using a hemocytometer and Reichert fluorescent microscope with a 490 nm excitation filter, a 500 nm dichroic filter, and a 515 nm emission filter (FITC filter combination). Acridine orange is a DNA intercalating dye and stains DNA and RNA only. Acridine orange fluorescent staining was essential to visualize leukocytes among the contaminating red blood cells, which otherwise interfere under a light microscope. The fluorescent dye was not used in counting Molt-4 cells because there was no red blood cell interference.

Table 1 The antioxidant capacity of freeze-dried (FD) and oven-dried (OD) leaves of *A. annua* from a cloned Swiss cultivar and of Brazilian and Chinese *A. annua* leaf ethanolic (EtOH) extracts determined by the oxygen radical absorbance capacity (ORAC) assay. All plants were field-cultivated in Beaver, WV, USA. They are expressed in micromoles of Trolox equivalents per gram of dry weight (µmoles TE/g DW). Total antioxidant capacity (TAC) of FD and OD leaves is the summation of the hydrophilic fraction of the leaves ($ORAC_{Hydro}$) and the lipophilic fraction ($ORAC_{Lipo}$). The $ORAC_{Lipo}$ of the ethanolic extracts was not determined (ND) and the TAC of the polar ethanolic extracts is equal to the hydrophilic fraction ORAC ($ORAC_{Hydro}$). Relative standard deviation (RSD) is shown in between parentheses.

Organ/ extract type	Hydrophilic $ORAC_{Hydro}$ (µmoles TE/g DW)	Lipophilic $ORAC_{Lipo}$ (µmoles TE/g DW)	TAC $ORAC_{Hydro + Lipo}$ (µmoles TE/g DW)
Leaves (FD)	1029.2 (15.8)	58.6 (23.0)	1088
Leaves (OD)	1041.7 (6.3)	39.6 (14.9)	1081
Brazilian EtOH extract	2535 (0.84)	ND	2535 (0.84)
Chinese EtOH extract	1960 (1.07)	ND	1960 (1.07)

FD = freeze-dried; OD = oven-dried; EtOH = 70% ethanol; ORAC = oxygen radical absorbance capacity; Hydro = hydrophilic fraction of sample submitted to ORAC assay; Lipo = lipophilic fraction of sample submitted to ORAC; TAC = total antioxidant capacity is the sum of $ORAC_{Hydro}$ plus $ORAC_{Lipo}$. Values are in µmoles of Trolox equivalents/ gram of sample dry weight (µmoles TE/g DW)

Data analysis

Each experiment was conducted three times using Molt-4 cells and leukocytes. Mean and standard deviation were calculated for each data point. Data were plotted as percentage, determined by dividing the mean cell count at 24, 48, or 72 hr by the mean cell count at 0 hr.

Data were analyzed by one-way ANOVA, and differences between pairs of data points were compared by the Newman-Keuls Test. Statistical difference was considered significant when $p < 0.05$.

Results

The antioxidant capacity of both Chinese and Brazilian extracts, determined by the ORAC assay, established that both extracts had high antioxidant capacity. However, the antioxidant capacity of the Brazilian ethanolic extract was approximately 30% higher than that of the Chinese cultivar. The total antioxidant capacity (TAC) of oven-dried Brazilian *A. annua* leaves (used to make the ethanolic extracts tested) was similar to the TAC of the freeze-dried subsamples (1081 and 1088 µmoles TE/g), with the hydrophilic fraction of the oven-dried Brazilian *A. annua* accounting for 96% of the total leaf antioxidant capacity, while lipophilic extracts accounted for only 4% (1041 µmoles TE/g DW vs. 39.6 µmoles TE/g DW, ◉ **Table 1**). While the TAC of the Brazilian ethanolic extract is higher than that of the Chinese extract, the concentration of artemisinin in the Chinese extract (2.7% in g/100 g DW) was approximately 2.2 times higher than in the Brazilian (1.26%) extract, although both were extracted in 70% ethanol in the same way.

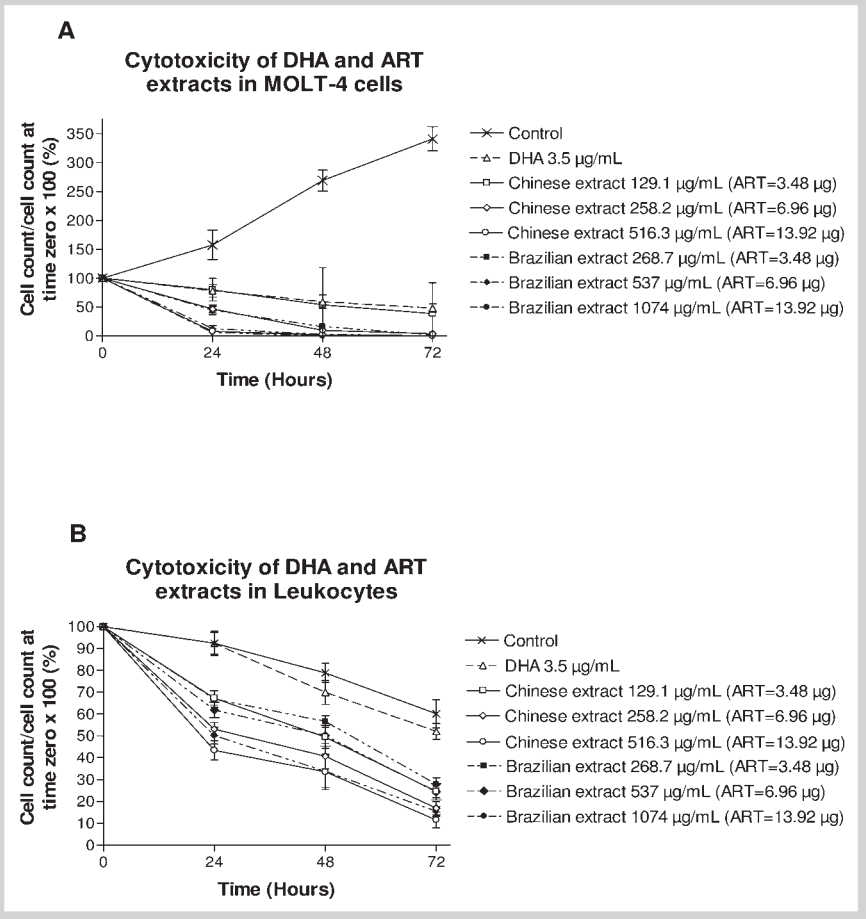

Fig. 2 Cytotoxicity of DHA and *Artemisia* (ART) ethanolic extracts (Brazilian and Chinese) on Molt-4 cells (**A**) and leukocytes (**B**) at various time points (24, 48, and 72 hr). The y-axis represents the percent cell count at time zero (%). Each point represents the mean of three experiments and the error bars represent the standard deviations of means. Final concentration of DMSO in all cultures including extract-free DMSO controls was 1%. Note: Concentration given for the extracts in artemisinin equivalents (µg/mL) were calculated from the quantification of artemisinin (in g/100 g DW) in the extracts based on HPLC-UV analysis.

The HPLC-UV chromatogram shows that the ethanolic extracts had most peaks (except the peak at 14 min) present in *A. annua* commercial extracts made with nonpolar hexane or petroleum ether (**Fig. 1**). The Chinese ethanolic extract also contained 1.6 times more dihydroartemisinic acid (1.46% vs. 0.9%, respectively), and 2.4 times more artemisinic acid (0.24% vs. 0.1%, respectively) than the Brazilian ethanolic extract (in g/100 g DW).

Fig. 2A and **B** show the cytotoxicity of various doses of DHA and *A. annua* extracts, as a percent cell count from time zero, in Molt-4 cells and leukocytes, respectively at 24, 48, and 72 hr time points. The results established that DHA and various doses of extracts are significantly effective ($p < 0.01$) in killing Molt-4 cells compared to extract-free DMSO controls.

As shown in **Table 2**, the relative toxicity (LD_{50} values) of each treatment on normal versus cancer cells was analyzed based on the mean of the cell count. At 48 hr of treatment, DHA had 1.26 times higher anticancer activity than the Brazilian ethanolic extract ($p < 0.05$) in killing Molt-4 cells. Moreover, at 72 hr of treatment, DHA was 1.66 times more potent than the Chinese extract ($p < 0.01$) and 1.49 times more potent than the Brazilian extract ($p < 0.01$). On the other hand, data from leukocytes showed that both Brazilian and Chinese extracts were more toxic to normal cells than DHA at 24 hr of treatment ($p < 0.05$), but the difference in toxicity between DHA and the extracts decreased considerably at 48 hr. Overall, no significant effect was observed between the two *Artemisia* extracts at all time points on Molt-4 cells and leukocytes.

Table 2 The LD_{50} (mean ± SD) of DHA, Chinese ethanolic extract, and Brazilian ethanolic extract on Molt-4 cells and leukocytes at various time points. Data used for LD_{50} calculations were averaged from three experiments. Asterisks indicate statistically significant difference from DHA. There were no significant differences between the two extracts at any time point in either cell type.

LD_{50} (µg/mL ART)	Cell type	24 hr	48 hr	72 hr
DHA	Molt-4 cells	6.38 ± 6.37	1.09 ± 0.06	0.87 ± 0.04
	Leukocytes	760.42 ± 493.99	61.81 ± 53.97	35.15 ± 30.33
Chinese	Molt-4 cells	3.00 ± 1.16	1.63 ± 0.42	1.44 ± 0.18*
Extract	Leukocytes	13.79 ± 4.38*	8.84 ± 3.58	2.83 ± 0.45
Brazilian	Molt-4 cells	1.81 ± 0.19	1.37 ± 0.01*	1.30 ± 0.01*
Extract	Leukocytes	28.23 ± 12.90*	14.05 ± 4.82	3.97 ± 1.08

Discussion

The Chinese ethanolic extract was 68% more concentrated in artemisinin than the Brazilian extract. This reflects the genotypic variation of seed-generated plants and between cultivars, which agrees with reports of different chemotypes for *A. annua* [18]. However, the commercial-type (petroleum ether) extract from a Brazilian plant had a similar phytochemical profile as both ethanolic extracts (**Fig. 1**) indicating that 70% ethanol can extract almost all components extracted by nonpolar solvents, including dihydroartemisinic acid and artemisinic acid. These compounds may synergize with artemisinin increasing its anticancer effects. It is also true that polar fractions, extracted in 70% acetone, usu-

ally retain 95% or more of the total antioxidant capacity compared to nonpolar fractions extracted in hexane [12] (**Table 1**). Thus, ethanolic extracts have higher concentrations of antioxidant flavonoids than hexane or petroleum ether extracts, increasing the likelihood of synergism for ethanolic extracts. Data also showed that the oven-dried leaves used to make the ethanolic extracts tested on Molt-4 cells had similar antioxidant capacity (hydrophilic ORAC) as freeze-dried subsamples, and that hydrophilic fractions contained over 95% of the total antioxidant capacity (TAC), while lipophilic antioxidant components of the extract (such as vitamin E) accounted for less than 4% of the TAC (**Table 1**). These ORAC results and the HPLC-UV data (**Fig. 1**) suggest that 70% ethanol is a perfect medium to extract most of the antioxidant components and artemisinin from *A. annua* leaves.

To evaluate the anticancer potential of *A. annua* extracts, we assessed the cytotoxicity of both dihydroartemisinin (DHA) and the plant extracts. DHA (an analog of artemisinin but not produced by the plant *A. annua*) was used primarily as an index and confirmation that our system of testing cytotoxicity was reliable. We were unable to use artemisinin as a marker because it is poorly soluble, making it unsuitable for this use, alongside liquid extracts. Interestingly, the LD_{50} value in Molt-4 cells for the Brazilian ethanolic *A. annua* extract was 3.5-fold lower than for DHA and 1.7-fold lower than for the Chinese extract at the 24 hr time point, while Brazilian and Chinese extracts were more toxic to leukocytes than DHA. The Brazilian extract was used at approximately twice the concentration of the Chinese extract to maintain the artemisinin equivalence in both extracts. The Brazilian extract was relatively more toxic to cancer cells than the Chinese extract, and both extracts were more toxic to normal cells than DHA (**Table 2**). Although our *in vitro* toxicity data indicated that both Chinese and Brazilian extracts were significantly more toxic to leukocytes than DHA (**Table 2**), animal studies showed that both extracts presented no toxicity when fed to gerbils by oral gavage at 100 mg/mL, for 5 consecutive days [19]. The dose used in that study was approximately 100–200-fold (100 mg/mL) higher than the maximum concentrations of the Chinese (516 µg/mL) and Brazilian (1074 µg/mL) extracts used here.

Several factors may be responsible for the significantly different effects of DHA and Brazilian ethanolic *A. annua* extract on cancer cells. While there are few studies comparing *A. annua* extracts with its synthetic derivatives (none for cancer), previous research on other natural compounds may elucidate this effect. It has been shown that isolating and using a pure active compound may do more harm than good since isolated components may lead to increased side effects as compared with whole plant extracts [20, 21]. Moreover, Olsson and collaborators [22] reported that pure vitamin C and purified compounds from whole fruit and berry extracts were not as effective in killing breast (MCF-7) and colon cancer (HT29) cells *in vitro* as crude extracts. These extracts had natural vitamin C and a higher antioxidant activity than pure vitamin C, indicating synergism between vitamin C and other compounds from the whole extracts [22].

The work of others [11] showed that dichloromethane extracts from the same Brazilian cultivar had more methoxylated flavonoids than a Chinese cultivar. They reported that these flavonoids were also found in hexane extracts and that they enhanced the reaction between artemisinin and hemin, synergizing artemisinin as antimalarial. If some of these flavonoids were also extracted in 70% EtOH at 60 °C, they could also have synergized the anticancer effect of artemisinin in the extract. This potential synergism has been recently reviewed for several flavonoids present in *A. annua* [23].

Although flavonoids have anticancer effects, they also carry some degree of toxicity to normal cells [24, 25]. This toxicity may be considerably lower *in vivo* than *in vitro* due to detoxification mechanisms not present in isolated cells. Potential benefits in the use of a flavonoid-rich extract include suppression of CYP450 enzymes, such as CYP2B6 and CYP34A, which degrade artemisinin, thus increasing artemisinin bioavailability [26]. Artemisinin alone has anticancer effects *in vitro* by suppressing nitric oxide synthesis through the inhibition of the nuclear factor NF-κB [27]. Thus, the potent anticancer activity of the ethanolic extracts could be due to artemisinin plus various compounds in the extract synergizing the artemisinin effect. Since flavonoids have been hypothesized to trigger internal antioxidants (such as glutathione) and blockage of cellular inflammatory responses instead of exerting a direct antioxidant activity, a plant extract may provide longer lasting changes than the direct effect of artemisinin alone. Furthermore, extracts may be effective in smaller doses, with fewer side effects [28]. Flavonoids have also been shown to suppress P-glycoproteins (ABC transporters involved in the multidrug resistant pumps found in cancer cells and in some parasites), which may explain why there are no records of resistance to whole plant extracts [29].

Because different extraction solvents and procedures may result in different contents of artemisinin and other anticancer agents, it is important to optimize the extraction of anticancer compounds from *A. annua* leaves and to standardize these extracts before further anticancer tests *in vivo*. Once extract preparation is optimized, a method of delivery with appropriate and safe solvents may lead to a plant extract with similar, or better anticancer efficacy than currently available chemotherapies, and with significantly less toxicity to normal cells. The results presented here warrant further research focused on elucidating the natural compounds responsible for the antioxidant capacity, cytotoxicity, and synergistic anticancer activities of *A. annua* ethanolic extracts.

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Conflict of Interest

The authors declare no conflict of interest. Mention of trade names or commercial products in this publication is solely for the convenience of the reader and does not imply endorsement of the U.S. Department of Agriculture over similar products. The USDA is an equal opportunity provider and employer.

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