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Effect of leaf digestion and artemisinin solubility for use in oral consumption of dried *Artemisia annua* leaves to treat malaria

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Abstract

Ethnopharmacological relevance—*Artemisia annua* L. produces the antimalarial sesquiterpene lactone, artemisinin (AN), and was traditionally used by the Chinese to treat fever, which was often caused by malaria.

Aim of the study—To measure effects of plant-based and dietary components on release of artemisinin and flavonoids from *A. annua* dried leaves (DLA) after simulated digestion.

Materials and methods—Simulated digestion was performed on DLA in four types of capsules, or in conjunction with protein, and protein-based foods: dry milk, casein, bovine serum albumin, peanuts, peanut butter, Plumpy'nut[®], and *A. annua* essential oils. Artemisinin and total flavonoids were measured in the liquid phase of the intestinal stage of digestion.

Results—After simulated digestion, peanuts and Plumpy'nut[®] lowered AN and flavonoids, respectively, recovered from the liquid digestate fraction. None of the compositions of the tested capsules altered AN or flavonoid release. Surprisingly, bovine serum albumin (BSA) increased both AN and flavonoids recovered from liquid simulated digestate fractions while casein had no effect. AN delivered as DLA was about 4 times more soluble in digestates than AN delivered as pure drug. Addition of a volume of essential oil equivalent to that found in a high essential oil producing *A. annua* cultivar also significantly increased AN solubility in simulated digestates.

Conclusion—These results indicate encapsulating DLA may provide a way to mask the taste of *A. annua* without altering bioavailability. Similarly, many peanut-based products can be used to mask the flavor with appropriate dosing. Finally, the essential oil fraction of *A. annua* contributes to the increased AN solubility in DLA after simulated digestion. Our results suggest that use of DLA in the treatment of malaria and other artemisinin susceptible diseases should be further tested in animals and humans.

Graphical Abstract

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¹Author contributions: MRD conducted all experiments and analyzed data; MRD and PJW designed experiments and wrote the manuscript.

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Keywords

malaria; bioavailability; flavonoids; essential oils; sesquiterpenoids (from JP list) artemisinin; digestion; pACT (ours additional)

Introduction

In 2015, there were 214 million cases of malaria resulting in 438,000 deaths worldwide (WHO, 2016). Of these deaths, 91% occurred in sub-Saharan Africa and 70% of the victims were children under 5 (WHO, 2016). Since 2000, malaria incidence and death rates have decreased globally by 37% and 60%, respectively, however progress has been slower in sub-Saharan Africa (WHO, 2016). The foremost therapeutic used to quell malaria worldwide is artemisinin (AN, Figure 1), but due to poor solubility, AN semisynthetic derivatives, e.g. artesunate, dihydroartemisinin and artemether, are the preferred drugs. AN is a naturally occurring sesquiterpene lactone produced and stored in the glandular trichomes of the plant *Artemisia annua* L. (Ferreira and Janick, 1995). AN derivatives are combined with other antimalarials, e.g. artemether + lumefantrine (Coartem[®]) to slow the evolution of AN resistance, and termed artemisinin combination therapies (ACTs), and are recommended by the WHO for treatment of malaria (WHO, 2015). Although ACTs are accepted as the frontline treatment for malaria, they are often too expensive or unavailable to those in need (Davis et al., 2013; Kyaw et al., 2014; Yeung et al., 2008). Indeed the highest malaria mortality rates occur in regions of the world with the highest proportions of people living on < \$1.25/day (WHO, 2012).

Recently, use of dried leaves of *A. annua* (DLA; aka pACT) to treat malaria has shown promise. This generally recognized as safe (GRAS) medicinal plant (Duke, 2001) has been used since 168 BCE in traditional Chinese medicine to treat a variety of conditions including “fever”, which was likely caused by malaria (Cui and Su, 2009). Traditionally the plant was prepared as a tea infusion however, this mode of preparation is not recommended as it is difficult to control the many parameters, such as temperature and time, which dictate phytochemical extraction and stability (van der Kooy and Verpoorte, 2011; Weathers and Towler, 2012). Instead, per os (p.o.) consumption of DLA should be used to ensure administration of a consistent dose. In a rodent study, p.o. DLA delivery of AN was compared to p.o. delivery of pure AN (Weathers et al., 2011), and DLA provided ~ 45 times

more AN in the serum than delivered from similar doses of pure AN. Furthermore, delivery as DLA was five times more effective at reducing parasitemia than pure AN in mice infected with *Plasmodium chabaudi* (Elfawal et al., 2012) and three times more resilient against emerging AN drug resistance (Elfawal et al., 2015).

A. annua is also rich in a variety of other compounds including flavonoids, phenolic acids, terpenes, coumarins, saponins, and essential oils (Elford et al., 1987; Ferreira et al., 2010; Lehane and Saliba, 2008; Suberu et al., 2013; Van Zyl et al., 2006). Many of these compounds have weak activity against malaria (Weathers and Towler, 2014) and some have been shown to synergize with AN (Liu et al., 1992; Suberu et al., 2013). For these reasons this plant-based artemisinin combination therapy (pACT) may provide an effective, inexpensive treatment option for those in extreme poverty.

One drawback to using DLA is the bitter taste associated with the dried leaves. Although preliminary data suggested about 61% of humans find the leaves distasteful, others actually like the taste (Supplemental Table 1). Nevertheless, masking the unpalatable taste with readily available food items or by encapsulation is desirable, especially for pediatric patients. Encapsulation or alternative taste masking is only feasible, however, if the capsules or foodstuffs do not significantly alter the bioavailability of the therapeutic compounds. In this study, we use a simulated human digestion system to investigate how various food items, pure proteins, and capsules affected AN and flavonoid content of intestinal stage digestates. We also used simulated digestion to investigate the solubility of AN in intestinal stage digestates when digested as pure drug vs. DLA. Our results also suggest a possible partial mechanism for the increased bioavailability of AN when delivered as DLA vs. as pure drug.

Methods

Plant material

Two *Artemisia annua* L. clonal cultivars propagated by rooted cuttings (Towler and Weathers, 2015; Weathers and Towler, 2012) were used in this study: SAM (DLAS) (voucher MASS 00317314), a high AN-producing cultivar (~1.4% w/w), and GLS (DLAG) (vouchers OR State Univ 171772 and 170353), a glandless AN-null mutant cultivar with no glandular trichomes that produces no AN (Duke et al., 1994) and 25% of the flavonoids found in SAM. SAM plant material used in protein and dietary constituent experiments was field grown in Stow, MA, harvested at floral budding stage, dried and processed as previously described (Weathers et al., 2014b). SAM plant material used in solubility experiments was grown in the lab under glass-filtered sunlight, harvested at the vegetative stage, dried, and processed same as the field grown SAM. GLS, a gift from Dr. Stephen Duke at University of Mississippi, was grown in the lab, under glass-filtered sunlight, harvested in the vegetative stage, dried, and processed same as SAM.

Chemicals and capsules

Chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified. Toluene was purchased from Thermo Fisher Scientific (Waltham, MA,

USA) and *A. annua* essential oils from Bella Mira (Mannford, OK, USA). Vegetable capsules made from hydroxypropyl methylcellulose (HPMC) and water and gelatin capsules made from beef gelatin and water were purchased from Capsule Connection LLC (Prescott, AZ, USA). Vcaps[®], Vcaps Plus[®], and Plantcaps[™] were a gift from Capsugel (Morristown, NJ, USA). Vcaps[®] are made of HPMC and a proprietary combination of gelling agents while Vcaps Plus[®] are made without the gelling agents. Plantcaps[™] are made from pullulan, a polysaccharide polymer fermented from tapioca. All capsules were size “00.” Evaporated milk, smooth peanut butter, and plain unsalted peanuts were store brand purchased from a local Shaw’s Supermarket (Stow, MA USA). Plumpy’nut[®], made from peanut based paste, is a Ready to Use Therapeutic Food (RUTF; USAID 2015, <https://www.usaid.gov/what-we-do/agriculture-and-food-security/food-assistance/resources/ready-use-therapeutic-food> Accessed 12-28-15) for treating malnutrition. Plumpy’nut[®] is produced locally by Edesia (Providence, RI, USA) and was a gift from Maternova Inc. (Providence, RI, USA).

Simulated digestion

Simulated digestion was performed according to Weathers et al. (2014a) (Figure 2). All digestions were taken to the intestinal stage before being vortexed and filtered through Whatman #1 chromatography paper (0.16 mm thickness, porosity < 10 µm) to separate solid and liquid digestate fractions. Liquid fractions were extracted in a sonicating water bath for 30 minutes with an equal volume of toluene to yield a clear two-phase separation to extract AN and flavonoids for analysis. Artemisinin but not all flavonoids are extracted by toluene. This solvent was required to obtain good phase separation especially for the oily materials, peanuts and Plumpy’nut[®], thus we kept the extraction solvent constant for all experiments in the study. AN and flavonoids found in the liquid fraction were of interest because they were in solution and thus more likely to diffuse across the intestinal wall into the blood (Weathers et al., 2014b). Extracts were dried under nitrogen gas and stored at –20°C until analysis. All digestions with capsules were performed with 2 capsules and all digestions with food items were performed with equal weights of food and plant material.

Solubility experiments

To determine the difference in solubility when AN was delivered orally as pure drug vs. as DLA, we performed simulated digestions of AN and of DLA and then captured unsolubilized AN on a filter and measured the soluble and insoluble fractions as subsequently described. A 0.18 g sample of sieved DLAS and an amount of AN equivalent to that measured in 0.18 g DLAS for that specific experiment were separately digested using the simulated digestion method of Weathers et al. (2014a). We also performed simulated digestions using a pure AN control and an equivalent amount of AN added to 0.18 g dried GLS leaves, which have no detectable AN. Finally, we performed simulated digestion of pure AN as a control and pure AN in combination with camphor (3.8 mg), quercetin (0.6 mg), or 0.55 and 7.2 µL of *A. annua* essential oil added directly to the simulated digestion. Camphor and quercetin amounts were chosen based on total amount of the monoterpene camphor or total flavonoids, respectively, as typically measured in DLAS. Essential oil values were chosen based on the reported extremes of essential oil in *A. annua* (Bilia et al., 2014). All simulated digestions were taken to the intestinal stage. After digestion, the digesta were filtered through Whatman #1 chromatography paper (0.16 mm thickness,

porosity < 10 μm) to separate liquid and solid fractions. Both fractions were then extracted with toluene and the extracts dried under nitrogen gas and prepared for analysis. AN extracted from liquid fractions was considered soluble and likely to be more bioavailable as particles <10 μm are absorbed more readily by intestinal epithelium (Desai et al., 1997). AN present in the solid fraction was considered insoluble or absorbed to residual plant solids and less likely to cross the intestinal wall. The percent AN dissolved was determined by dividing the amount of AN found in the liquid fraction by the total AN recovered. AN solubility ($\mu\text{g/mL}$) was calculated by dividing the total AN recovered from the liquid digestate fraction by the volume.

Artemisinin and flavonoid analysis

AN in extracts was quantified using GCMS according to the method detailed in Towler and Weathers (2015). Flavonoids in extracts were quantified using the spectrophotometric AlCl_3 method (Arvouet-Grand et al., 1994) with quercetin as a standard. Flavonoid levels are expressed as quercetin equivalents.

Statistical analysis

All experiments were performed in at least triplicate. Students T-tests and One-way ANOVA tests were used to determine statistical significance ($p < 0.05$) where appropriate and Kruskal-Wallis tests were used whenever there were unequal numbers of control and experimental samples. The statistical program GraphPad Prism 6 was used to perform all statistical analysis.

Theory

In malaria endemic countries, cheaper and readily available antimalarials are needed for people in extreme poverty. DLA may be able to fill this niche, but masking the bitter taste would make delivery simpler for the mostly young children who need it. Using a simulated digestion method, we tested masking agents and capsules for their effect on the levels of therapeutic compounds released into the intestinal digesta. This method also enabled measurement of AN solubility post simulated digestion, which will improve our understanding about DLA enhancement of AN bioavailability. Understanding the factors that dictate bioavailability of AN and flavonoids from DLA provides useful knowledge for clinicians.

Results and Discussion

Capsules do not alter AN or flavonoid content of digesta

When DLA was run through the simulated digestion with capsules, none of the tested capsules altered the amount of AN extracted from the intestinal liquid (Figure 3A). Previously we reported that vegetable and gelatin capsules negatively affected the release of AN from DLAS after simulated digestion (Weathers et al., 2014b). That study used methylene chloride to extract the digestate and under some conditions there was not a well-defined phase separation. In this study, however, we used toluene as an extraction solvent instead of methylene chloride and obtained a well-defined two-phase separation. None of the

tested capsules showed a significant difference in total flavonoid content of liquid digestate fractions (Figure 3B). Since capsules are designed to be chemically inert, these results should be expected. These results suggested that encapsulation may provide a simple means of masking the bitter taste of DLA without altering drug bioavailability. Capsule selection now can be based mainly on cost instead of performance.

Some food items alter AN and flavonoid content of digesta

Another option for masking the taste of DLA is to combine it with common dietary constituents found in sub-Saharan Africa. This serves a dual purpose by not only providing an antimalarial therapeutic, but also a nutritious supplement. As malnutrition exacerbates malaria and other diseases, especially in low income countries (Caulfield et al., 2004), this is desirable. Previously we showed that some dietary constituents commonly used in sub-Saharan Africa altered the AN and flavonoids released into liquid fractions of simulated digestions (Weathers et al., 2014b). These food items were mainly simple and complex carbohydrates and various oils. Previously, however, there were reports that AN bound to serum proteins (Bian et al., 2006; Liu, R. et al., 2014) and there was concern that protein in the diet may decrease AN bioavailability during digestion. Thus, we performed simulated digestions with several protein-rich dietary constituents to determine if they would alter AN content of the liquid digestate fraction. After simulated digestion of DLAS + peanuts, the AN content in the liquid digestate fraction decreased by about 23% (Figure 4A, $p = 0.028$). Dry powdered milk, smooth peanut butter, and Plumpy'nut[®], however, had no effect on AN content of the liquid digestate fraction. There was also a significant increase in AN released after digestion of DLAS + bovine serum albumin (BSA) (Figure 4C $p = 0.006$). AN is known to bind BSA (Bian et al., 2006; Liu, R. et al., 2014) and is sometimes used in drug absorption studies to bind free drug and maintain sink conditions (Hubatsch et al., 2007), so it is conceivable that the BSA interacted with free AN in the digestate solution allowing more AN to be extracted from the solid DLAS fraction into the liquid DLAS fraction of the digestate. Casein, the main protein found in milk, did not have any effect. It therefore seems unlikely that proteins are responsible for major decreases in AN content in liquid digestate fractions. The mechanism by which peanuts and not peanut butter decreased AN content is still unresolved.

Besides AN, we also tested the effects of these same dietary constituents on total flavonoid content in liquid digestate fractions. After digestion, total flavonoids decreased in the liquid fraction by about 24%, and only in the presence of Plumpy'nut[®] (Figure 4B, $p = 0.003$). However, in the presence of BSA, flavonoids increased by about 56% (Figure 4D, $p = 0.0015$) in the liquid digestate fraction. Similarly to AN, casein had no effect. BSA has been shown to also strongly bind flavonoids (Bi et al., 2012; Liu, S. et al., 2014; Papadopoulou et al., 2005), so the observed increase in flavonoids is likely due to the same mechanism described for AN wherein BSA creates stronger sink conditions allowing more flavonoids to be extracted from the solid fraction DLAS into the liquid digestate. While the differences in AN and flavonoid content in the presence of peanuts and Plumpy'nut[®], respectively, were statistically significant, neither therapeutic compound is decreased enough to discourage their use as a masking agent. Rather, if using either peanuts or Plumpy'nut[®] to mask DLA flavor, then the amount of DLA could be proportionately increased to ensure adequate

delivery to yield a minimum therapeutically effective serum concentration of $9 \mu\text{g L}^{-1}$ (Alin and Bjorkman, 1994).

Delivery method changes AN solubility

AN has significantly higher bioavailability when delivered p.o. as DLA as opposed to pure AN (Weathers et al., 2011; Weathers et al., 2014a). However, the mechanism by which AN bioavailability is so greatly enhanced is yet to be uncovered. We posited that AN solvates better when combined with some other phytochemicals found in the plant material. To test this hypothesis, we carried out simulated digestions of either pure AN or an amount of DLAS containing the same amount of AN. After digestion, it was determined that the liquid digestate from DLAS contained about four times more AN than the pure AN liquid digestate fraction (Table 1, $p = 0.005$). This large increase in AN solubility partially explains the increased bioavailability seen when AN is delivered orally as DLAS in mice (Weathers et al., 2011). To query which compounds or groups of compounds were responsible for the increase in AN solubility we first performed simulated digestions with addition of pure AN and equal amounts of pure AN added to 0.18g DLA of the GLS cultivar (DLAG). GLS lacks glandular trichomes and thus produces no detectable AN, only 0.06% (w/w) essential oils (Tellez et al., 1999), and only 25% of the flavonoids of SAM, so we could determine if the increased AN solubility was due to general plant matrix or some specific groups of chemicals exclusive to one of the two cultivars. After simulated digestion of DLAG + AN and pure AN, there was no significant difference in the amount of AN in the liquid digestate fractions (Table 1). These results indicated that the compounds responsible for increasing AN solubility are present in DLAS but not in the GLS cultivar.

Essential oils increase AN solubility

The essential oil composition of *A. annua* cultivars ranges from 0.3–4.0% (w/w) (Bilia et al., 2014). Since the essential oil content of DLAS is unknown, we tested both high and low levels of essential oil for its effect on AN solubility in digestates. Aliquots of *A. annua* essential oil equivalent to 0.3 and 4% of 0.18 g of DLAS dry mass were added to simulated digestions with pure AN. After the intestinal stage of digestion, there was about 2.5 times more AN dissolved in the liquid digestate fraction of the 4% essential oil group (Table 1, $p = 0.004$). No significant difference was found between the amount of solubilized AN in the pure AN control and 0.3% essential oil + AN groups (Table 1). Thus essential oils in the plant are likely in part responsible for the increased solubility of AN when delivered p.o. as DLAS as opposed to pure AN. We also tested the hydrophobic flavonoid, quercetin, and a principal component of the hydrophobic essential oil, camphor, to determine if they played a role in increasing AN solubility. While both of these hydrophobic compounds have low aqueous solubility at the start of simulated digestion, the changes in temperature and pH, as well as the addition of bile salts in the intestinal phase likely allows some to become emulsified. Bile is a lipid emulsifier in digestion that allows the breakdown of lipids into micelles so they can be readily absorbed by the intestine. After digestion to the intestinal stage, quercetin did not alter AN solubility suggesting hydrophobic flavonoids play no role in increasing the solubility of AN delivered as DLAS. Interestingly, the hydrophobic monoterpene, camphor, also had no effect on AN solubility in digestates suggesting that it is likely other compounds present in the essential oil that lead to enhanced AN solubility.

Conclusions

Determining how digestion effects AN and flavonoid content in the intestine after oral drug delivery is paramount to ensuring proper dosing and understanding the differences between DLAS and pure AN. Using a simulated digestion method, we showed that peanuts decreased AN content in liquid digestate fractions by 23%. Although peanut butter was benign, Plumpy'nut[®], a peanut-based RUTF used to treat malnutrition in sub-Saharan Africa, decreased flavonoid content by 24% in liquid digestate fractions. As these dietary constituents decrease the levels of therapeutic compounds delivered by DLA, those recommending DLA as a treatment for malaria should consider altered dosing if these food items are used to mask the bitter taste. Since none of the tested capsule types decreased either AN or flavonoid content, capsules offer an acceptable means of masking the unpalatable flavor of DLA.

Using this simulated digestion method we were also able to partially explain the mechanism by which DLA enhances AN bioavailability in prior rodent studies. In simulated digestions AN delivered via DLAS yielded four times more AN in the liquid digestate fraction than pure AN, indicating some compounds in the SAM cultivar enhanced AN solubility. This was not true for AN delivered with dried leaves of the GLS cultivar, likely the result of the dearth of essential oils in GLS. Furthermore, AN delivered in combination with essential oil from *A. annua* at a volume consistent with a high essential oil producing plant showed a 2.5 fold increase in AN solubility suggesting essential oil plays a role in the increased solubility afforded by DLAS. Together these results provide insight into how DLAS might function and be used as an inexpensive yet still effective alternative to traditional ACT medication for malaria.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Alin MH, Bjorkman A. Concentration and time dependency of artemisinin efficacy against *Plasmodium falciparum* in vitro. *The American journal of tropical medicine and hygiene*. 1994; 50(6):771–776. [PubMed: 8024073]
- Arvouet-Grand A, Vennat B, Pourrat A, Legret P. Standardization of propolis extract and identification of principal constituents. *Journal de pharmacie de Belgique*. 1994; 49(6):462–468. [PubMed: 7884635]
- Bi S, Yan L, Pang B, Wang Y. Investigation of three flavonoids binding to bovine serum albumin using molecular fluorescence technique. *Journal of Luminescence*. 2012; 132(1):132–140.

- Bian H, Li M, Yu Q, Chen Z, Tian J, Liang H. Study of the interaction of artemisinin with bovine serum albumin. *International journal of biological macromolecules*. 2006; 39(4):291–297. [PubMed: 16730793]
- Bilia AR, Santomauro F, Sacco C, Bergonzi MC, Donato R. Essential oil of *Artemisia annua* L.: an extraordinary component with numerous antimicrobial properties. *Evidence-Based Complementary and Alternative Medicine*. 2014; 2014
- Caulfield LE, Richard SA, Black RE. Undernutrition as an underlying cause of malaria morbidity and mortality in children less than five years old. *The American journal of tropical medicine and hygiene*. 2004; 71(2 suppl):55–63. [PubMed: 15331819]
- Cui L, Su X-z. Discovery, mechanisms of action and combination therapy of artemisinin. 2009
- Davis B, Ladner J, Sams K, Tekinturhan E, de Korte D, Saba J. Artemisinin-based combination therapy availability and use in the private sector of five AMFm phase 1 countries. *Malar J*. 2013; 12(135):10–1186. [PubMed: 23298401]
- Desai MP, Labhasetwar V, Walter E, Levy RJ, Amidon GL. The mechanism of uptake of biodegradable microparticles in Caco-2 cells is size dependent. *Pharmaceutical research*. 1997; 14(11):1568–1573. [PubMed: 9434276]
- Duke, JA. Handbook of phytochemical constituents of GRAS herbs and other economic plants. CRC Press LLC; Boca Raton, FL: 2001.
- Duke MV, Paul RN, Elsohly HN, Sturtz G, Duke SO. Localization of artemisinin and artemisitene in foliar tissues of glanded and glandless biotypes of *Artemisia annua* L. *International Journal of Plant Sciences*. 1994; 155(3):365–372.
- Elfawal MA, Towler MJ, Reich NG, Golenbock D, Weathers PJ, Rich SM. Dried whole plant *Artemisia annua* as an antimalarial therapy. *PLoS ONE*. 2012; 7:e52746. [PubMed: 23289055]
- Elfawal MA, Towler MJ, Reich NG, Weathers PJ, Rich SM. Dried whole-plant *Artemisia annua* slows evolution of malaria drug resistance and overcomes resistance to artemisinin. *Proceedings of the National Academy of Sciences*. 2015; 112(3):821–826.
- Elford BC, Roberts MF, Phillipson JD, Wilson RJM. Potentiation of the antimalarial activity of qinghaosu by methoxylated flavones. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1987; 81(3):434–436. [PubMed: 3318019]
- Ferreira JFS, Janick J. Floral morphology of *Artemisia annua* with special reference to trichomes. *International Journal of Plant Sciences*. 1995:807–815.
- Ferreira JFS, Luthria DL, Sasaki T, Heyerick A. Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. *Molecules*. 2010; 15(5): 3135–3170. [PubMed: 20657468]
- Hubatsch I, Ragnarsson EGE, Artursson P. Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. *Nat Protocols*. 2007; 2(9):2111–2119. [PubMed: 17853866]
- Kyaw SS, Drake T, Ruangveerayuth R, Chierakul W, White NJ, Newton PN, Lubell Y. Cost of treating inpatient falciparum malaria on the Thai-Myanmar border. *Malaria journal*. 2014; 13(1):416. [PubMed: 25351915]
- Lehane AM, Saliba KJ. Common dietary flavonoids inhibit the growth of the intraerythrocytic malaria parasite. *BMC Research Notes*. 2008; 1(1):26. [PubMed: 18710482]
- Liu KCSC, Yang SL, Roberts MF, Elford BC, Phillipson JD. Antimalarial activity of *Artemisia annua* flavonoids from whole plants and cell cultures. *Plant Cell Reports*. 1992; 11(12):637–640. [PubMed: 24213368]
- Liu R, Cheng Z, Jiang X. Comparative studies on the interactions of dihydroartemisinin and artemisinin with bovine serum albumin using spectroscopic methods. *Luminescence*. 2014; 29(8): 1033–1046. [PubMed: 24804646]
- Liu S, Guo C, Guo Y, Yu H, Greenaway F, Sun MZ. Comparative Binding Affinities of Flavonoid Phytochemicals with Bovine Serum Albumin. *Iranian journal of pharmaceutical research: IJPR*. 2014; 13(3):1019. [PubMed: 25276204]
- Papadopoulou A, Green RJ, Frazier RA. Interaction of flavonoids with bovine serum albumin: a fluorescence quenching study. *Journal of agricultural and food chemistry*. 2005; 53(1):158–163. [PubMed: 15631523]

- Suberu JO, Gorka AP, Jacobs L, Roepe PD, Sullivan N, Barker GC, Lapkin AA. Anti-Plasmodial Polyvalent Interactions in *Artemisia annua* L. Aqueous Extract – Possible Synergistic and Resistance Mechanisms. *PLoS ONE*. 2013; 8(11):e80790. [PubMed: 24244716]
- Tellez MR, Canel C, Rimando AM, Duke SO. Differential accumulation of isoprenoids in glanded and glandless *Artemisia annua* L. *Phytochemistry*. 1999; 52(6):1035–1040.
- Towler MJ, Weathers PJ. Variations in key artemisinin and other metabolites throughout plant development in *Artemisia annua* L. for potential therapeutic use. *Industrial Crops and Products*. 2015; 67:185–191. [PubMed: 25729214]
- van der Kooy F, Verpoorte R. The Content of Artemisinin in the *Artemisia annua* Tea Infusion. *Planta Med*. 2011; 77(15):1754–1756. [PubMed: 21544776]
- Van Zyl RL, Seathholo ST, Van Vuuren SF, Viljoen AM. The biological activities of 20 nature identical essential oil constituents. *The Journal of Essential Oil Research*. 2006; 18:129–133.
- Weathers PJ, Arsenault PR, Covello PS, McMickle A, Teoh KH, Reed DW. Artemisinin production in *Artemisia annua*: studies in planta and results of a novel delivery method for treating malaria and other neglected diseases. *Phytochemistry Reviews*. 2011; 10(2):173–183. [PubMed: 21643453]
- Weathers PJ, Elfawal MA, Towler MJ, Acquah-Mensah GK, Rich SM. Pharmacokinetics of artemisinin delivered by oral consumption of *Artemisia annua* dried leaves in healthy vs. *Plasmodium chabaudi*-infected mice. *Journal of ethnopharmacology*. 2014a; 153(3):732–736. [PubMed: 24661969]
- Weathers PJ, Jordan NJ, Lasin P, Towler MJ. Simulated digestion of dried leaves of *Artemisia annua* consumed as a treatment (pACT) for malaria. *Journal of Ethnopharmacology*. 2014b; 151(2):858–863. [PubMed: 24316176]
- Weathers PJ, Towler MJ. The flavonoids casticin and artemetin are poorly extracted and are unstable in an *Artemisia annua* tea infusion. *Planta Medica*. 2012; 78(10):1024–1026. [PubMed: 22673829]
- Weathers PJ, Towler MJ. Changes in key constituents of clonally propagated *Artemisia annua* L. during preparation of compressed leaf tablets for possible therapeutic use. *Industrial crops and products*. 2014; 62:173–178. [PubMed: 25228784]
- WHO. Factsheet on the World Malaria Report 2012. 2012. <http://www.who.int/malaria/media/world_malaria_report_2012_facts/en/>
- WHO. Guidelines for the treatment of malaria. 3. World Health Organization; Geneva, Switzerland: 2015.
- WHO. Malaria Fact Sheet. 2016. <<http://www.who.int/mediacentre/factsheets/fs094/en/>>
- Yeung S, Van Damme W, Socheat D, White NJ, Mills A. Cost of increasing access to artemisinin combination therapy: the Cambodian experience. *Malaria journal*. 2008; 7(1):84. [PubMed: 18492245]

Figure 1.
Structure of artemisinin (AN).

Figure 2.
Schematic for simulated digestion method.

Figure 3. Artemisinin (A) and flavonoid (B) content in liquid fraction from intestinal stage of simulated digestions of DLAS done $\pm$ various capsule types. N = 3; *, p < 0.05 compared to AN control within each experiment.

Figure 4. Artemisinin (A) and flavonoid (B) content in liquid fraction from intestinal stage of simulated digestions of DLAS ± various protein-rich dietary constituents. Artemisinin (C) and flavonoid (D) content in liquid fraction from intestinal stage of simulated digestions of DLAS ± BSA. N = 3; *, p < 0.05 compared to AN control within each experiment.

Table 1

Effects of different delivery methods on AN content in liquid simulated digestate fractions from four independent experiments.

Experiment	Treatment	AN fold change in digestate liquid fraction
Pure AN vs. DLAS	AN	1.00a
	DLAS	4.08b
Pure AN vs. AN + DLAG	AN	1.00a
	AN + DLAG	0.91a
Pure AN vs. AN + Essential Oils	AN	1.00a
	AN + 0.3% EO	0.95a
	AN + 4.0% EO	2.46b
Pure AN vs. AN + Camphor or Quercetin	AN	1.00a
	AN + Camphor	1.09a
	AN + Quercetin	0.89a

DLAS and DLAG, 0.18 g dry weight. Values normalized to AN control; n = 3; a,b letters show p < 0.05 compared to AN control within each experiment.