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**ARTEMISIA ANNUA L.: THE HOPE AGAINST MALARIA AND
CANCER**

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Index:

1. Malaria and cancer.
2. Brief history on *A. annua*.
3. Production *in vivo* and *in vitro*.
4. Morphological aspects of artemisinin accumulation.
5. Artemisinin analysis.
6. Resurgence of traditional use.
7. Present and future of artemisinin.
8. Conclusions.
9. References

1. Malaria and cancer:

Malaria is a major scourge of humankind which continues to defy science and technology. Malaria afflicts ca. 500 million people a year worldwide, killing approximately 2 million of the people who live in endemic regions, which is 41% of the world population (Martens and Hall, 2000). Most victims of malaria are children from the sub-Saharan Africa, but victims are also counted all over the tropical world. Although malaria has been eradicated from the US and Europe, it still claims approximately 1,500 cases a year in the US and a few other cases in Europe, mainly around international airports. Malaria is transmitted by *Anopheles* mosquitoes, and has the potential to cause outbreaks in the US, where the mosquito vector is found in all 48 continental states. Malaria has been slowly and steadily coming back, and is developing global resistance against two of the most common antimalarial drugs: chloroquine and the combination sulphadoxine/pyrimethamine (Ridley, 2002).

Cancer is a general term used to describe the uncontrolled proliferation of somatic cells, which can develop into a tumor or spread around the body affecting one or more vital systems. In the United States alone, cancer kills over 500 thousand people a year, being the second killer after heart disease. Among the types of cancer, lung cancer kills the most, indistinctive of sex, followed by prostate cancer in man and by breast cancer in women. According to Brown (1990), cancer is an economic burden to the United States, costing over 585 million just in health care, not to mention disability and productivity losses. Traditional treatment includes radiation therapy or chemotherapy; the latter uses synthetic or semi-synthetic drugs that kill cancer cells as well as healthy cells. The chemotherapy treatment also causes undesirable side

effects such as loss of appetite, nausea, and depression. Thus, the need for a medicine that is affordable and less toxic to healthy cells and for the individual cannot be overstressed.

2. Brief history of *A. annua*.

Artemisia annua (Asteraceae) is native to China, where it is known as qinghao (green herb) and has been used for over 2,000 years to treat symptoms associated with fever and malaria. It is known in the United States as sweet Annie, annual or sweet wormwood (Ferreira et al., 1997). The name of the plant is derived from that of the Greek goddess Artemis, daughter of Zeus, twin sister of Apollo, and credited with “healing diseases and averting evil”. In antiquity, plants of the genus *Artemisia* were used to control the pangs of childbirth, to regulate women’s menstrual disorders, and as an abortifacient (Riddle and Estes, 1992). In 1969, the Chinese screened their medicinal plants in search of an effective antimalarial. A diethyl ether extract of *A. annua* was found to be effective against *Plasmodium* sp., and in 1972 the active ingredient, artemisinin, was isolated and identified by the Chinese. From approximately 400 species of artemisias, *A. annua* is the main source of artemisinin. Artemisinin can be synthesized *de novo* in the lab, but the low yield and multi-step procedure makes the plant the only feasible source of the artemisinin.

3. Production *in vivo* and *in vitro*.

Artemisinin can be produced both *in vivo* and *in vitro* cultures, as long as the cultures are allowed to differentiate. Although artemisinin has been reported from transformed root (Rao et al., 1998, Cai et al., 1995) and shoot cultures (Paniego and Giulietti, 1996), the best *in vitro* sources of artemisinin are whole plant cultures or shoot cultures, which bear glandular trichomes (Ferreira and Janick, 2002). Rao and collaborators (1998) reported that their transformed root cultures only produced artemisinin when transferred from dark to light (16 h/day) conditions, suggesting that photosynthesis and thus chloroplasts are involved in the biosynthesis of artemisinin. Artemisinin was found only in traces or missing from callus and cell cultures, and although whole-plant cultures have artemisinin in levels similar to greenhouse and field cultures, the maintenance of plants under *in vitro* conditions for extended periods has been shown to decrease artemisinin production or to make the selection process unreliable. Also, when differentiated shoot cultures were treated with hormones that increased shoot growth but decreased root growth, artemisinin levels decreased as the number of shoots increased (Ferreira and Janick, 1996). Clones kept *in vitro* for two years correlated poorly in artemisinin with the same clones kept under greenhouse conditions (Ferreira et al. 1995b).

4. Morphological aspects of artemisinin accumulation.

Artemisinin was found to be absent from roots and pollen, but present in main stems, side stems, leaves, and flowers of *A. annua*. Artemisinin has been linked to organs that bear glandular trichomes. These trichomes have been characterized in the leaves by Duke and Paul (1993) and in the flowers by Ferreira and Janick (1995), and are believed to be the specific site of artemisinin biosynthesis and sequestration. The

presence of bitter terpenes, such as artemisinin, in glandular trichomes is believed to protect the plant from being grazed upon by herbivores.

5. Artemisinin analysis.

Artemisinin is a sesquiterpene lactone with an unusual peroxide bridge, which is the key moiety in the eradication of *Plasmodium falciparum* and the elimination of breast cancer cells. Artemisinin has been quantified in the past by various analytical methods such as TLC, HPLC-UV after derivatization to a compound which absorbs at 260 nm, HPLC-EC, GC-MS, GC-FID, and ELISA. These methods were recently reviewed by Christen and Veuthey (2001). However, all methods have presented limitations such as the cost of the analytical equipment, such as GC/MS and HPLC/MS. Other methods such as HPLC with electrochemical detection (HPLC-EC) are specific for artemisinin and the compounds that have the peroxide bridge (dihydroartemisinin, artemisitene, arteether, artelinate, artemether). However, HPLC-EC does not detect precursors of artemisinin such as arteannuin B and artemisinic acid or compounds that have only one oxygen instead of the peroxide bridge (deoxyartemisinin). Another limitation of HPLC-EC is the trouble in setting up the method without the interference of oxygen, which is a must because artemisinin is analyzed with the EC detector in the “redux” mode. Once the method is working, one still has to wait ca. two hours for the baseline to stabilize before any injection is done. These limitations also apply to the coulometric detection system.

High-performance liquid chromatography with evaporative light scattering detection (HPLC-ELSD) is resurging as an alternative method for the analysis of artemisinin and derivatives because it detects compounds with or without a peroxide bridge. It analyzes the molecules as a whole and it is ready for injections within 15 minutes of turning on the detector.

Gas chromatography with flame ionization detection (GC-FID) has been excluded in the past due to the fact that it breaks down artemisinin in 2-3 different compounds, and does not separate one of the artemisinin break-down products from arteannuin B. However, with the recent advances in gas chromatography and stationary phases, it is currently possible to quantify artemisinin and its precursor by GC-FID without peak overlapping (unpublished data).

6. Resurgence of traditional use.

The price of artemisinin-derived drugs, when compared to quinine-derived drugs, has been labeled as “expensive”; something around \$6.00 for a whole treatment with artemisinin, compared to ca. \$0.50 for a treatment with quinine-derived drugs. However, when someone is afflicted with a life-threatening strain of *Plasmodium* that is resistant to quinine-derived and other drugs, six dollars is all that stands between life and death. One has to keep in mind that quinine-derived drugs are produced synthetically, but the only commercial source of artemisinin is *Artemisia annua*. Although *A. annua* has been used for ca. 2000 years by the Chinese, artemisinin has been around for only 32 years, while quinine has been used for ca. 300 years. Compared to other pharmaceutical drugs, artemisinin is not expensive. In reality, one of the commercial producers of artemisinin stated that there was no profit in selling artemisinin in malaria-afflicted countries. The main goal achieved by producing the

drug was to have their company's name associated with the life-saving antimalarial. In the future, this would bring buyers to trust and seek their other products.

Currently, there is a trend in using medicinal plants the traditional way. There are news of multi-drug resistant *Plasmodium falciparum* that is nonetheless susceptible to extracts of the *Cinchona* tree. Apparently, other natural antimalarials, present in the bark's extract, such as quinidine, have a synergistic effect, potentiating the effects of quinine. The same is believed to be true for artemisinin. Some authors have reported that *A. annua* extracts are three times more effective than artemisinin by itself, considering the levels of artemisinin in the plant extract (Willcox et al., 2004). Also, the presence of other compounds in the extract might make it harder for the *Plasmodium* to mutate around the mixture than around artemisinin used alone.

7. Present and future of artemisinin.

Artemisinin low yields in the lab point to *Artemisia annua* as the natural and feasible source of artemisinin. While chemists search for new, more stable, less toxic semi-synthetic artemisinin drugs, the commonly used antimalarials (dihydroartemisinin, artesunate, artemether, and sodium artelinate) all depend on natural artemisinin as the raw material.

One of the most recent uses of artemisinin-related drugs is the treatment of cancer. The combination of dihydroartemisinin with ferrous sulfate have been reported to reduce tumor growth (Moore et al., 1995). Recently, Singh and Lai (2001) demonstrated that dihydroartemisinin was specifically toxic to breast cancer cells, but not to healthy cells. More extensive research showed that another artemisinin drug (artesunate) was also effective against several types of cancer (Efferth et al., 2001).

Artemis®, a new cultivar has been selected that produce close to 1% artemisinin (Delabays et al., 1993). This late flowering clone is suitable to tropical areas and has been tested in Switzerland (Debruner et al., 1996), in Brazil (Magalhães et al., 1997), in Africa (Mueller et al., 2000), and in the United States (Ferreira, unpublished). Artemis® clones reach their peak in artemisinin during the vegetative stage as opposed to clones used in the past by Ferreira and collaborators (1995a), which reached their peak in artemisinin production only during full flowering. It is also known that artemisinin is controlled mainly by genetic factors, according to broad-sense heritability (Ferreira et al., 1995b) and narrow-sense heritability (Delabays et al., 2001) studies. Thus, clones selected for high artemisinin can be vegetatively propagated and distributed to achieve a stable and predictable artemisinin yield by populations in need. The future of a stable source of artemisinin, until feasible *de novo* synthesis is possible, is certainly the production through *Artemisia annua*. Some groups are now trying to understand the biosynthesis of this sesquiterpene lactone in the plant, while other researchers have succeeded in producing one of artemisinin early precursors (amorpha-4,11-diene) in *E. coli* (Wallaart et al., 2001).

8. Conclusions.

Currently, there is an urgent need for antimalarial drugs that are affordable, effective against susceptible and multi-drug resistant malaria, and that have low toxicity. One of the most commonly used antimalarials (mefloquine) has been

associated with unpleasant side effects such as vomiting, diarrhea, and even depression and suicide. However, with the exception of suicide, the side effects are always better than dying of malaria when visiting the tropics. Artemisinin-derived drugs have low toxicity because they target protozoan cells that are loaded with iron, acquired through the *Plasmodium* feeding on hemoglobin. Thus, while the long expected malaria vaccine does not become a reality, artemisinin is our best hope against multi-drug resistant malaria caused by *Plasmodium falciparum*. It is important to highlight that any use of artemisinin to treat malaria, even through *Artemisia annua* extracts, must be followed by a physician to prevent the appearance of strains of *Plasmodium falciparum* resistant to this new line of antimalarial drug.

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