

Artemisia annua; the plant, production and processing and medicinal applications.

by

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Abstract

**The plant:** This section contains the taxonomy, common names, a description (morphology, anatomy and physiology), ecology (habitat, environment, distribution, pollination, services and status), pollination, ethnobotany, notes and a bibliography.

**Production and processing:** This deals with the production (areas and demand, markets and economics), cultivation (systems, land, multiplication, planting, water, fertility, weeding and harvest), improvement (genetic resources, varieties, breeding and biotechnology), products and uses (processing, characteristics, uses) notes, pest notes and a bibliography.

**Medicinal applications:** The pharmacopoeial name is given followed by uses (parts used, preparation, constituents, standards, methodology), pharmacology (systems, ailments, clinical trials, quality control, precautions, toxicology), wild harvesting (methodology, legislation, conservation), marketing, cultivation, notes and a bibliography.

Finally there is a list of entities, references and glossary terms mentioned in the text.

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## Introduction

*A. annua* is a vigorous, annual, aromatic, herbaceous plant reaching 1-3 m height and 1 m in width. It is a crop for the production of anti-malarial and possibly antibacterial agents and natural pesticides. It was originally collected by the Chinese as an herbal medicine and is

currently processed by pharmaceutical firms for the production of artemisinin for Artemisinin-based Combination Therapies (ACTs) in the treatment of malaria. ACTs have been shown to have; rapid resolution to fever and parasitaemia; low toxicity and are well tolerated. The artemisinin compounds are effective against *Plasmodium falciparum* and *P. vivax*, including multi-drug-resistant strains.

## 1.0 The Plant

This section contains the taxonomy, common names, description (morphology, anatomy and physiology), ecology (habitat, environment, distribution, pollination, services and status), pollination, ethnobotany, notes and a bibliography.

### 1.1 Taxonomy

The taxonomy of *Artemisia annua* according to Mabberley D.J. 1997. Lineage: Class: Magnoliopsida, Subclass: Asteridae, Order: Asterales, Family: Compositae, Genus: *Artemisia*, Specific epithet: *annua*.

#### 1.1.1 Compositae

Genera/Species: 1528/22750.

Distribution: Cosmopolitan (except Antarctica).

Habit: Shrubs and herbs (especially rhizome), trees and climbers, often storing carbohydrate as polyfructosans (especially inulin); articulated laticifers (mostly Lactuceae) or more or less extensive resin duct system present; secondary thickening well developed even in many herbaceous subspecies., sometimes in unusual configurations or with medullary and/or cortical bundles.

Leaves: Spirally arranged, less often opposite, rarely whorled, simple, dissected or more or less compound; stipules 0.

Flowers: Inflorescences of 1 numerous dense heads (capitula) with 1 - numerous sessile flowers on common receptacle, nearly always subtended by an involucre of 1 several series of bracts (0 in *Psilocarphus*), opening in racemose sequence (mixed in *Espeletia*); capitula occasionally aggregated into cymose secondary heads (over 40 genera in several tribes) sometimes with a secondary involucre; receptacle flat to conical or cylindrical, sometimes with a bract subtending each flower (especially *Helianthea*) or bristly (especially *Cardueae*). Flowers epigynous, bisexual or some female, sterile or functionally male (radiate heads with marginal female or sterile ray florets and central bisexual or functionally male disk florets, ray florets with tubular corolla prolonged into more or less strap shaped ligule often tipped with vestigial 3 corolla lobes (other 2 more or less absent), disk florets with regular tubular (4)5 lobed or toothed corolla; discoid heads with only disk florets; disciform heads with central disk florets and marginal female florets with eligulate corolla, or with only the latter; ligulate heads (mostly Lactuceae) of bisexual florets with corolla of 5 lobes; in *Mutisieae* some or all florets with 2 lipped corolla, outer larger but marginal florets sometimes as in Lactuceae and some or all of central ones like disk florets); calyx forming pappus on top of ovary or 0, of (1)2 numerous scales, awns, bristles or connate to form crown, stamens as many as corolla lobes, alternate with them and inserted in tube, usually distinct, anthers usually with short apical appendage and sometimes basal tails and pollen sacs connate into tube, releasing pollen

into tube through longitudinal slits to be pushed out by growth of style, inferior gynoecium (2), 1-locule with terminal style, bifid, branches commonly separating after passage through anther tube; nectary commonly a thickened scale or cup on top of gynoecium (nectar usually amino acids and hexoses), ovule 1, basal, erect, anatropous, unitegmic.

Fruits: A cypsela usually with persistent pappus, rarely a drupe.

Seeds: Embryo oily, straight with hypocotyl, endosperm 0 or very thin peripheral. Basic chromosome number = 2-19+ (? orig. 9). V.H. Heywood et al. (1977) *Biology and chemistry of the Compositae.*; K. Bremer (1994) *Asteraceae*; Journal: *Compositae Newsletter*.

Classification and large genera: (Bremer):

I. Barnadesioideae

II. Cichorioideae

III. Asteroideae

Most conspecies in diversity in montane subtropical and tropical areas with a tendency for tribal and subtribal specialization in ecology and habit, though within particular genera there may be great variation e.g. *Coreopsis* and *Erigeron* with aquatics to xerophytes; *Blepharispermum*, *Brachylaena*, *Eremanthus* and *Vernonia* include timber trees and *Piptocarpha* dominates the seasonally burnt landscapes of East Central Brazil. The capitulum in animal pollinated subspecies acts in many ways like a single flower (compare *Aizoaceae*), the long tubed flowers of *Carclueae* being visited by bees and *Lepidoptera*, the yellow and white flowers so common in the rest of the family being attractive to flies, beetles etc. At anthesis the pollen is forced out by the stigma, the floret being functionally male, later the stigmatic arms separate such that the floret is functionally female; often the stigmas then curl back to touch the pollen on the style thus effecting self fertilization. A number of genera especially *Hieracium* and *Taraxacum* have large numbers of apomictic lines ('microspecies'). Wind pollinated occurs in *Ambrosia*, *Artemisia* etc. It has been customary to argue that the ecological success in terms of species and individual numbers of the family is due to the capitulum system, the involucre acting like a calyx, the fruit wall like a testa, etc., but the pseudanthial head is 'duplicated in the small and unsuccessful family *Calyceraceae*' (Cronquist A, 1978) while a similar pollen presentation mechanism is found in *Brummia* (*Goodeniaceae* in the broad sense; flowers in cymose heads) and developed to varying degrees in *Campanulaceae*, other *Goodeniaceae* and part of *Rubiaceae*. Cronquist A, 1978 attributes the success of the C. to the defensive combination of polyacetylenes and the bitter sesquiterpene lactones followed by the development of other chemical repellents like the alkaloids in *Senecioneae*, the latex system of *Lactuceae*, which do not have the polyacetylene bearing resin system of the other tribes; the bad smell of the *Tagetes* group of *Helenieae* and the characteristic smells of *Anthemideae* are such repellents: many of these compounds make the plants important as sources of flavourings and insecticides.

Recent cladistic and molecular work suggests the *Compositae* are closely allied to *Calyceraceae*, with which they share similar corolla venation, and *Goodeniaceae* all placed in an expanded *Asterales* with *Campanulaceae* and its allies (see *American Journal of Botany* 82(1995)250). Contrary to received doctrine, the family is probably primitively woody (compare *New Phytologist* 73(1974)967) and perhaps of South America and Pacific origin (though paucity in New Caledonia difficult to explain and fossils from mid Jurassic China

claimed), the early members being pachycaul with large discoid capitula of yellow flowers and involucre bracts in several rows

Use: Compared with other large families, e.g. Gramineae, Leguminosae, the Compositae are of little value to Man except as ornamentals, the edible ones having low levels of toxins or, as in lettuce, having had them selected out: some are insecticides and fish poisons but many are noxious weeds, their fruit spread by wind (pappus) or animals (sticky pappus in *Adenostemma*, pappus of barbed bristles in *Bidens*, involucre bracts with hooked tips in *Arctium*, sticky bracts in *Sigesbeckia*, receptacle with hooks in *Xanthium*, etc.). With increasing clearance of native vegetation throughout the world, these aggressive toxic plants will inherit it.

### 1.1.2 *Artemisia*

About 350 species North temperate (Europe 55; China 170), West South America, South Africa (1); usually dry areas. Reference: CN 25(1994)39. Aromatic shrubs and herbs (ragweeds): vermifuges stimulants, culinary herbs, cultivated ornamentals characteristic of Russian steppes. See also *Seriphidium*. Capitula small, wind pollinated, causing hay fever problems (in United States 1 sq. mile emits 16 t in 2 weeks (August - September). *Artemisia abrotanum* L. (southernwood, lad's love, old man, origin unknown) - very rarely flowering, medicinal, also tea; *Artemisia absinthium* L. (absinthe, wormwood, Europe) - a ketone (thujone) medicinal, plant pest control since time of Pliny (AD 77) and absinthe (harmful liqueur); *Artemisia afra* Jacq. ex Willd. (tropical Africa to East Cape) - local medicinal; *Artemisia annua* L. (Eurasia, naturalized North America) - efficacious antimalarial (huanghuahaosu) in China, due to artemisinin, (sesquiterpene lactone) also phytotoxic, leaves used for burns; *Artemisia arborescens* L. (Medit.) - hedgeplant in New Zealand; *Artemisia cina* Berg ex Polj. (Levant wormseed, Turkestan) - medicinal, anthelmintic (santonin) known as santonica; *Artemisia dracunculoides* L. (tarragon, estragon, Eurasia) - leaves for flavouring (terpineol), especially terpineol vinegar used with fish; *Artemisia glacialis* L. and *Artemisia umbelliformis* Lam. (*Artemisia laxa*, Alps) flavouring for genepi liqueur; *Artemisia herba-alba* Asso (Med.) wormwood of the Bible; *Artemisia lactiflora* Wallich (China) - cultivated ornamentals herb. perennial; *Artemisia pontica* L. (Roman wormwood, South East Europe) - flavour of vermouth; *Artemisia tilesii* Ledeb. (Arctic) - medicinal (Esquirnaux), properties like codeine; *Artemisia vulgaris* L. (rue, wormwood, Eurasia) - leaves a condiment, also used in magic and superstition in Great Britain until 19 Century.

### 1.1.3 Common Names

qinghaosu, jing hao, ching hao, qing hao, 菊科植物□花蒿, qing hao su (Chinese), sweet annie, wormwood, sweet wormwood, sweet sagewort, annual mugwort, annual wormwood (English), kesämaruna, rikkamaruna (Finish), armoise (French), wermut, einjähriger beifuss (German), losna doce (Portuguese), ajenjo (Spanish), sommarmalört (Swedish).

### 1.2 Description

It is a vigorous, erect, annual, (sometimes bi-annual), aromatic, herbaceous plant reaching 120-200 cm or even up to 300 cm in height and 100 cm in width. Non-glandular T-shaped trichomes and 10-celled biserial glandular trichomes occur on leaves, stems, and especially inflorescences. There is strong evidence that artemisinin is sequestered in these glandular trichomes.

### 1.2.1 Morphology

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**Roots:** It has a short taproot and aggressive fibrous roots.

**Stems:** It is usually single-stemmed, base up to 1 cm diameter, with many alternate branches. Stems are first light coloured at the top, later getting a reddish tint lower down and finally becoming deep reddish/purple. The stem has longitudinal green furrows when young, changing to deep reddish purple when branching occurs.

**Leaves:** The foliage is fernlike. The leaves are aromatic, alternate, 2.5-5.0 cm long, bright green, deep and fine pinnately dissected into very narrow short, obtuse 5-8 lobes and of a papery texture. These lobes are further dissected and have deeply dentate points 1-2 mm long and 0.5-1 mm wide. The central leaf vein is clear with slight apophysis and both sides have narrow dentate wings. The leaf base is widely olivaeform to triangularly olivaeform; 2.5-7 cm long and 2-6 cm wide and withers at florescence. On both sides of the leaf there are many 10-celled biseriate trichomes and 5-celled filamentous trichomes, which are easily detachable. Basal leaves have slender petioles, 1-2 cm long, middle leaves are 2-3 pinnate, glabrous and semi- amplexicaul while the upper leaves are sessile and less divided but none of them entire. When crushed the leaves have a refreshing slightly balsamic aroma.

**Flowers:** The tiny, numerous, globose, drooping or inclining flowers (capitula) - only 2-3 mm across are displayed in numerous loose, drooping panicles, about 2-3 cm wide, with short, slender peduncles containing many, greenish or yellowish, hermaphrodite disc florets containing little nectar and pistillate marginal (ray) florets. The involucre is imbricated with several rows (3-4) of linear bracts. The corolla is narrow and tubular and 3-4 split dentate at the brim and glandular trichomes outside. The stigma is linear and extends out of the corolla with 2 acute forks. (See diagram). The central flowers are perfect and can be either fertile or sterile. Flowers are first light yellow eventually becoming more golden. In the northern hemisphere it is in flower from August to September.

The pollen is tricolpate and smooth, typical of anemophilous species, and has vestigial or no spines. It has an internal, complex, columellae-tecta configuration in the exine, which is common to all taxa of the tribe Anthemideae and varies from two to three layers in *A. annua*. The pollen may be very allergenic.

**Fruits:** Ovaries are inferior and unilocular and each generates one achene, ca. 1 mm in length and faintly nerved. The pistillate marginal florets in the capitulum produce numerous achenes without pappus.

**Seeds:** Seeds are tiny, about 1 mm long, oblong, yellowish-brown with a lustrous surface marked by vertical furrows and with a creamy white fatty endosperm. The 1000 seed weight is about 0.03g, it re-seeds heavily. Seeds are not recalcitrant.

### 1.2.2 Anatomy

See the leaf cross-section (after photo by Marchese et al., 2005).

### 1.2.3 Physiology

*A. annua* is a determinate short-day plant. Non-juvenile plants are very responsive to photoperiodic stimulus and flower about two weeks after induction. The critical photoperiod seems to be about 13.5 hours, but there are likely to be photoperiod x temperature interactions.

Vietnam genotype clones of *A. annua* present a photoperiodic behaviour of Short-Day Plants (qualitative or absolute) with low temperature or vernalization requirement to have their flowering accelerated. The critical or inductive photoperiod ranges between 11 and 13 hours to Vietnam genotype and 13 and 15 hours to Chinese genotype. The Vietnam genotype plants flourish about 4-5 weeks after submitted to the inductive photoperiod, while the Chinese genotype plants flourish in 2 weeks. With regard to flowering, *A. annua* genotypes with different geographical origins can present variations in the behaviour under the same photoperiod and temperature conditions. (Marchese et al., 2002).

The existence of an evident parenchyma bundle sheath with little differentiation of the mesophyll cells may represent a transitional stage in the evolution of C4 photosynthesis pathway from C3 photosynthesis pathway. (Lawlor, 2001 and Condon et al., 2002), but, in general, the Kranz anatomy bundle sheath consists of a single layer of large cells containing large chloroplasts and starch. (Mauseth, 1988 and Rudall, 1994). The test with PAS produced a negative reaction for the vascular bundle sheath cells to starch, indicating the absence of chloroplasts in these cells. The results of  $\delta^{13}C$  together with the absence of chloroplasts and starch in parenchyma vascular bundle sheath cells suggest that leaves of *A. annua* has a C3 photosynthesis pathway. (Marchese et al., 2005).

The glandular trichomes are more prominent in the corolla and receptacles florets than in leaves, stems, or bracts. There is strong evidence that artemisinin is sequestered in these glandular trichomes, (Duke et al., 1994 and Ferreira and Janick, 1995). Although these glands are present since the early stage of development on both leaves and inflorescences, artemisinin increases at anthesis, suggesting that it accumulates as the glands reach physiological maturity, a stage which coincides with the end of cell expansion in floret development. As glands approach maturity, there appears to be a cellular discharge into the subcuticular space around the apical cells and the contents are spread over the epidermis when the glands burst. After anthesis, artemisinin decreases and so does the number of intact glands. The association of artemisinin with glandular trichomes sequestration explains why artemisinin was not detected in parts of the plant that do not bear glands, such as pollen or roots. (See Table: 'Artemisinin content of different organs and structures of greenhouse- and field- grown *Artemisia annua*, determined by HPLC-EC').

The leaves from the same plant may have different artemisinin contents, according to their localisation along the stem: upper leaves contain significantly more artemisinin than middle and lower ones. The plant content in artemisinin also varies during the season. Under Swiss conditions, highest artemisinin concentrations are found at the end of August, independently of the developmental stage of the plant (see Figure). Furthermore, the genetic basis and environmental factors such as temperature or nutrient availability further influence the artemisinin content in the plant. (Delabays et al., 2001).

Results from experimental fields of CIMAP, subtropical north India, show that the yield of oil obtained from leaves and inflorescence vary according to the season of cultivation. This indicates that, in the biosynthesis of the oil constituents of *A. annua*, temperature plays an important role. A significant difference in temperature of winter and monsoon months has been observed in the subtropical north Indian plains. The average temperature usually ranges between 10-26°C in the winter and 25-33°C during monsoon season. The results also show that as the plants matured, the oil content decreased in leaves from 0.32-0.14% and increased in inflorescence from 0.35-0.42% on a fresh weight basis. (Bagchi et al., 2001).

### 1.3 Ecology

It thrives in many temperate to sub-tropical ecologies. The plant is un-adapted to the tropics because flowering will be induced when the plants are very small; with the possible exceptions of high altitude plateaus and/or regions with a pronounced cool period such as Vietnam where there are two months of low temperatures at the beginning of the season. In tropical zones with less day length variation and shorter photoperiods overall, genotypes of *A. annua* will achieve only one meter or less in height before flowering and senescing, thus precluding production of sufficient biomass per hectare to yield sufficient extract per unit land area.

#### 1.3.1 Habitat

It can be found on barnyards, farm lots, fallow fields, borders of woods, mudflats along streams and ponds, waste ground, roadsides, railroads, alleys of older cities and in cultivated beds. It has become a weed in many areas of the world.

#### 1.3.2 Environment

Latitude: Found between 30°S to 60°N.

Altitude: Occurs naturally as part of a steppe vegetation in the northern parts of Chahar and Suiyuan provinces in China, at 1000-1500 m above sea level. (Duke, 1983). Generally found between sea level and 3600 m.

Temperature: Reported temperature range for growth is 10-35°C with the optimum between 13-29°C. Winter hardy down to about -15°C. It is an annual plant with a growth cycle of about 180 days. (in cultivation 80 days in the nursery and 100 days in the field).

Water: Reported annual rainfall range for growth is 300-1500 mm with the optimum between 500-1300 mm. Grown as a field crop it requires an annual rainfall of at least 600-650 mm. It can withstand dry conditions once established, but any moisture stress in the early stages tends to induce flowering and reduce production of leaves.

#### Radiation

Range & intensity: It prefers a sunny position. (Chittendon, 1951 and Huxley, 1992). But it can also grow under light shade.

Photoperiodism: It is a determinate short-day plant. Non-juvenile plants are very responsive to photoperiodic stimulus and flower about two weeks after induction. The critical photoperiod seems to be about 13.5 hours, but there are likely to be photoperiod x temperature



interactions. In Lafayette Indiana, USA (40°21'N) plants flower in early September with mature seeds produced in October. (Duke, 1983).

#### Soil:

Physical: It prefers light to medium textured soils (sandy and loamy soils). It requires well drained or dry soils and thrives in fertile soils but will grow in nutritionally poor soils. (Plants for a Future, 2000). Plants are more aromatic when grown in a poor dry soil. Often found on low and alluvial ground and on mudflats. The plants are longer lived, more hardy and more aromatic when they are grown in a poor dry soil. (Genders, 1994).

Chemical: Prefers circumneutral or slightly alkaline soil. Reported soil pH range for growth is 5.5-8.5 with the optimum between 6-8.

#### 1.3.3 Distribution

It is native to Asia, the center of origin is most probably China; wild populations occur both in China and Vietnam. It has become naturalized in many countries including Argentina, Bulgaria, France, Hungary, Romania, Italy, Spain the United States, and the former Yugoslavia etc. It is widely dispersed throughout the temperate regions of the world.

It has been introduced for cultivation to Brazil, Cameroon, Ethiopia, India, Kenya, Mozambique, Myanmar, Tanzania, Thailand, Uganda, Zambia etc. - all in high-altitude regions and/or regions with a pronounced cool period.

Bhakuni et al., 2001 state that it is cropped on a large scale [also] in Afghanistan, Australia, Iran and Turkey. (Unconfirmed). Research work has been done in Australia (e.g. Laughlin, 1992) and in Turkey (e.g. Mert et al., 2002). Laughlin (pers. com. Aug. 2005) confirms that only areas of about one hectare were on state research farms but not on commercial farms as such. Prof. Ms. Filiz Ayanoglu (pers. com. Sept. 2005) confirms that it is not cultivated in Turkey.

#### 1.3.4 Pollination

The plant is anemophilous; naturally cross-pollinated by insects and wind action, which is unusual in the Asteraceae. (McVaugh, 1984).

#### 1.3.5 Services

Medicinal: The major service is in the production of artemisinin used in the treatment of malaria.

Environmental: Due to its aggressive fibrous root system it is used for erosion control on slopes. For example - on the limestone outcrops in the Chinese Autonomous Region.

IPM: There is potential for use in Integrated Pest Management. Where intercropped with fruit trees and maize in China, pesticide use has decreased. When intercropped with coffee in E. Africa, the incidence of coffee leaf miner is drastically reduced.

#### 1.3.6 Status

Genetic resources are threatened as harvesting of natural wild plant populations is done by cutting whole plants before flowering.

It has become introduced and naturalized in many countries; sometimes becoming invasive.

It is cultivated in many countries; but with a narrow genetic base.

As an annual crop it is cultivated in China and Vietnam as a source of artemisinin. Cultivated in Romania as a source of essential oils. Cultivated on small scale in the United States as a source of aromatic wreaths. (Janick, 1995).

#### 1.4 Ethnobotany

Etymology: Greek, after Artemis (to whom it was sacred). Artemis was Apollo's twin sister and daughter of Zeus and Leto; she was the Greek goddess Diana, goddess of the moon, the woods, and the wild, who, the legend states, derived so much good from plants of this kind that all such plants are named for her.

Uses:

Medicinal: Qing Ho or Ching-hao, better known in the West as sweet wormwood, is a traditional Chinese herbal medicine with a long history. The Pharmacopoeia of the People's Republic of China has registered its use for malaria, fever caused by tuberculosis, jaundice, fever caused by "summer heat" (hyperthermia) and afternoon fever related to "deficiency of yin". (Pharmacopoeia Commission, 2000). Symptoms of yin deficiency include afternoon or low grade fever, malar flush, dry mouth, insomnia with mental restlessness, anxiety, feverish sensations in the palms and soles, night sweats, constipation, concentrated urine, etc. The tongue becomes red with little coating and the pulse becomes rapid.

Ching-hao was described in the Shennong Bencao Jing where it was listed as caohao: Caohao is bitter and cold. It mainly treats skin bugs, itchy scabs, and malign sores; it kills lice, relieves lodged heat in the joints, and brightens the eyes. Its other name is qing hao.

Many other effects of *A. annua* products have been described in various sources: antiperiodic, antiseptic, digestive, febrifuge; an infusion of the leaves is used internally to treat fevers, colds, diarrhoea etc.; externally, the leaves are poulticed onto nosebleeds, boils and abscesses. (See "Links" icon - 'Plants for a Future'). Also - contraception, relief of joint pains, de-worming, haemorrhoids, immune boosting and cancer treatment.

Pharmacology:

The major recommended use is for the production of ACTs in the treatment of malaria. ACTs have been shown to have; rapid resolution to fever and parasitaemia; low toxicity and are well tolerated. The artemisinin compounds are effective against *P. falciparum* and *P. vivax*, including multi-drug-resistant strains.

It has been recommended for use in herbal tea infusions for treatment of malaria. However, from a medical viewpoint, based on current knowledge, the recommendation of artemisinin teas is not acceptable by WHO. As regards the development of resistance, some researchers argue that the presence of various substances in the aqueous extract will protect against resistance. One cannot exclude this possibility, but it is as yet unproven. There appears to be

only one peer-reviewed, published controlled clinical trial of artemisinin tea (Mueller et al., 2004) who state that monotherapy with *Artemisia annua* L. cannot be recommended as an alternative to modern anti-malarials, but may deserve further investigation. R  th et al., 2004 found that artemisinin plasma concentrations after intake of this herbal tea are sufficient for clinical effects, but insufficient to recommend such preparations as equivalent substitutes for modern artemisinin drugs in malaria therapy.

So far, apart from the anti-malarial effect the only use in humans supported by controlled clinical trials is the effect on schistosomiasis (Utzinger et al., 2003).

Condiment: An essential oil in the leaves is used as a flavouring in spirits such as vermouth. (Duke, 1983).

Religious: It is used in the crafting of aromatic wreaths.

1.5 Notes

Contacts:

This record is under construction (November 2005) in collaboration with WHO:.....

As well as various Internet sites consulted (see the "Links" icon) information has been supplied personally by Allan Schapira, WHO; James Simon, Rutgers/ASNAPP; James Duke, ARS; Barney Gasston, Africa Bio-Medica Ltd. & EABL; Qizhan Tang, Gaungxi Academy of Agricultural Sciences (GXAAS, China); Nguyen Van Thuan, Vietnam; Anil Singh, CIMAP, India; Jos   Abramo Marchese, Federal University for Technology of Paran  , Brazil; Pedro M. de Magalh  es, UNICAMP/CPQBA, Brazil; John Laughlin Tasmania, Australia; Dan Gudahl, Winrock International (re. Cameroon); Daw Khin Phyu Phyu, Myanmar; to be continued with more contacts and contact data.....

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2.0 Production and processing

This section deals with the production (areas and demand, markets and economics), cultivation (systems, land, multiplication, planting, water, fertility, weeding and harvest), improvement (genetic resources, varieties, breeding and biotechnology), products and uses (processing, characteristics, uses) notes, pest notes and a bibliography.

## 2.1 Production

See table for a provisional production area forecast (ha) of *A. annua* to 2007. (14550 ha). Vietnam, for example, presently cultivating 3000 ha, has satisfied local demand and has excess artemisinin for the world market.

Commercial production of *Artemisia* in Africa has largely been limited to Kenya and Tanzania. Current demand for artemisinin has led to a significant increase in the commercial production, both in established as well as new areas.

The demand for ACTs are growing and is expected to expand dramatically in 2005. WHO (2005) indicates that it increased from 2 million treatments in 2003 to 30 million in 2004 and a projected 70 million in 2005. This expansion has in turn led to a corresponding increase in demand for artemisinin. The price in China nearly quadrupled in the fall of 2004. (McNeil, 2005). In order to produce these quantities, immense volumes of raw material of *A. annua* are required. To spread the risk of climatic factors and disease that might impact the harvest of *A. annua*, it is important to diversify the geographic areas under cultivation. In addition, expanding cultivation and extraction capacity in Africa makes sense given the fact that most patients affected by malaria live on the African continent.

Leaf production is very variable depending on edapho-climatic conditions and agro-technology. In East Africa yields average 2.5 t/ha (range = 0.75-4.2). New agro-technologies; particularly harvesting techniques, can bring the potential to 8 t/ha. (EABL; pers. comm. from B. Gasston, 2005 and CIMAP; pers. comm. from Anil Singh, 2005).

Artemisinin content is also highly variable; in natural populations it may be as low as 0.01% and in improved cultivars as high as 1.3%. During extraction about 20% of this is lost. The range of E. African content by assay is 0.85-1.2%.

Artemisinin can also be obtained from artemisinic acid which occurs at concentrations as much as 10-fold higher than artemisinin. (Acton et al., 1985). Vonwiller et al., 1993 reported an extraction method which makes possible the extraction of both compounds from the same plant material, thus increasing the final production of artemisinin.

### Supposition:

Average global yield = 2 t/ha dried leaves.

Average artemisinin production (after extraction) = 0.6%

Thus - artemisinin yield = 12 kg/ha.

With 14550 ha = 174.6 t artemisinin in 2007.

This is below the WHO forecast for artemisinin supply. However, application of improved technologies will close this gap. For example the EABL agro-technology mentioned above; Chinese selections as opposed to wild material; and (for example) an improved agro-technology and molecular breeding technique for high yielding varieties has been developed at CIMAP, which are reported to produce over 60 kg/ha of artemisinin (extrapolation from

trial results; being field-tested by industry). In field cultivation carried out in Brazil, 3 hybrid lines produced respectively 21.38, 19.27 and 15.80 Kg of artemisinin/ha. (Magalhães et al., 2004).

### 2.1.1 Markets

Hundreds of millions of people at risk of drug-resistant falciparum malaria are in urgent need of access to quality artemisinin-based combination therapies (ACTs) at affordable prices. WHO forecasted in 2004 that the global demand in 2005 would be over 130 million adult treatment courses. In a later forecast (WHO; June 2005, Arusha Tanzania - to be published) the end of 2006 forecast is about 580 million.

The Institute of Medicine of the National Institutes of Health, USA, has proposed a global subsidy to ensure that ACTs are available and affordable everywhere; however, a number of issues must be clarified before such a subsidy could be implemented.

International cooperation is needed to solve these supply chain issues, until a stable market is established. This will require a comprehensive strategy including financial inputs, strengthened forecasting, scientific and technical inputs and collaboration between producers, consumers and partners.

WHO now recommends four artemisinin-based combination therapy (ACT)s: artemether-lumefantrine (Coartem®; Novartis), artesunate-mefloquine, artesunate-amodiaquine, and artesunate-sulfadoxine/pyrimethamine. Coartem dominates; Novartis has transferred IP rights to the Institute of Microbiology of the Academy of Military Medical Science in China for least developed countries; but for other countries the patent is held by Novartis until 2011.

### 2.1.2 Economics

Because of the shortage of artemisinin raw material, the artemisinin accounts for less than 1% in the international anti-malaria medicine market. (Zhou et al., 2005).

Field production of *A. annua* is presently the only commercially viable method to produce artemisinin because the synthesis of the complex molecule is uneconomic. (Janick, 1995).

Usually, only *A. annua* with an artemisinin content of over 0.6% has commercial value; although this is still rather low.

WHO and Novartis, the manufacturer of artemether/lumefantrine (Coartem®), have entered into a special pricing agreement: Novartis provides the drug at cost price (US\$ 0.9 and 2.4 per child and adult treatment course respectively) for use in the public sector in malaria-endemic countries.

The Global Fund to Fight Aids, Tuberculosis and Malaria (GFATM), established in 2002, is now the largest funder of ACTs in countries. In first three rounds of funding, a total of US\$ 41 million has been committed over the full 5-year life of GFATM Board-approved proposals for the purchase of ACTs in African countries. (World Bank, 2003).

Other sources of funds for ACT purchases available to countries include development banks, multilateral and bilateral agencies and NGOs.

One risk in relation to large-scale cultivation is absence of matching extraction capacity leading to wastage or use of the plant leaves for teas etc., which are not very effective for treating malaria. Even with adequate extraction, there is also a risk of production temporarily exceeding demand, which could lead to wide fluctuations of prices and output volumes.

## 2.2 Cultivation

GAP (Good Agricultural Practice) is the combination of Integrated Crop Management (ICM) techniques, minimum pesticide use and control of all facets of the production process to ensure the least possible impact on the environment and the provision of a product that can be accurately traced from the field where it is grown to the customer. By using GAP the aims of East African Botanicals Ltd. are to provide quality products to its customers while improving the environment and minimizing the risks of pesticide usage. As a good start to understand cultivation related to GAP; see the EABL Growers Production Manual for *A. annua* - April 2005.

### 2.2.1 Systems

**Minimal till systems:** Artemisia has been grown successfully under minimal till systems, the aim of which is to conserve soil moisture. Spraying off the vegetation 6 days before land preparation is essential to ensure weeds do not compete with the crop. The land can then be ripped to an appropriate depth, and the seedlings can then be planted by hand along the rip line, into prepared soil. Ripping will allow rainfall and dew to infiltrate the rip line, making it immediately available to roots. In some soils, when a very deep rip is made, it may be necessary to lightly roll the land afterwards in order to close the rip slightly, in order to reduce the risk that the seedling drowns in a heavy rainstorm.

**Intercropping:** Artemisia has also been successfully grown as an intercrop with coffee seedlings in Tanzania. When grown in one line between the coffee rows, there was no significant shading effect caused by the Artemisia. It was also observed that the Artemisia benefited the coffee by repelling leaf miner, as well as suppressing weeds in the coffee.

Artemisia is currently being grown as a trial in stumped coffee. EABL recommends that it be planted at one row per line between the coffee to avoid shading the coffee. This is an exciting trial, in which it may be shown that Artemisia helps to suppress weeds and pests, adds organic matter, and helps in soil erosion control, as well as being a cheaper method of production (stumped coffee must still be weeded, irrigated, and sub-soiled, therefore production costs are reduced, and Artemisia gives a return in an otherwise barren year for coffee).

For interplanting with coffee, EABL recommends that Artemisia be planted 1 m from each coffee seedling/stump, and approximately 1 m between each plant. This will result in a much lower population than usual, but will minimise shading coffee. (EABL, 2005).

### 2.2.2 Land

Good land preparation is critical, and it is important to note that short cuts designed to cut costs are seldom cost effective. Artemisia can have a deep taproot, and a mass of feeder roots, which gives the plant a good chance of surviving tough conditions once it is established. However, the seedling is very delicate in its initial stages, so good conditions are essential at transplanting.

The use of tined implements for cultivation is recommended. This conserves moisture in the soil, and can assist in weed control just before planting. Some soils of Kenya are known to have a natural hard pan or a compaction pan that has been created by earlier cultivation practices. If these occur, it is advisable to deep rip the soil to ensure good root penetration and water percolation through the soil profile. A deep rip is better than a shallow rip to allow maximum penetration.

Some soils may require the plants to be established on ridges. This will be necessary if furrow irrigation is practiced or where soils are heavy and prone to retaining excess water in the rain season. Ridges should be wide enough to maintain their shape after heavy rain and high enough to keep the main feeding roots free of any possible waterlogging. This generally means ridges of greater than 15 cm high and 20 cm wide but this will vary from soil-soil and on-farm experience will dictate the size of ridge used.

Start soil preparations well before the rain season to ensure that the soil is not cultivated when too wet as this will lead to loss of soil structure and compaction of the soil resulting in poor plant performance. Early preparation will also assist in weed control that is an extremely important factor in growing *Artemisia* successfully as weeds will germinate in between first and second cultivations. By destroying these with the second cultivation (ploughing or ridging) it will reduce the amount of time required for hand weeding.

If deep-rooted weeds such as perennial grasses are present it is recommended by EABL that glyphosate herbicide be applied at least 6 days before the initial cultivation. The rate used will depend on the species of weed present. Follow the instructions carefully as glyphosate applied in the wrong conditions will lead to poor results and difficult hand weeding conditions. (EABL, 2005).

#### Maintenance of soil structure

Some soils can be very fragile and good cultivation timing is essential. These soils are often lacking in organic matter and this is an important component in maintaining the structure under cultivation. If organic matter such as well rotted animal manure can be added to the soil it will materially assist in improving the structure, giving better aeration, greater root penetration and water percolation and greater efficiency in utilizing irrigation water. The addition of up to 20 tonnes per hectare of composted or well rotted manure is highly recommended for both a short term crop stimulant and as a long term component in an Integrated Crop Management programme to improve soil structure. (EABL, 2005).

#### 2.2.3 Multiplication

Propagation is mostly by seed. Vegetative propagation has been developed. Tissue culture uses standard protocols using shoot tips of mature field grown plants (Simon et al., 1990). Murashige and Skoog, 1962 medium enhanced with BA2 and NAA at 0.01 mg/ml was used by Dr. Chung Heon Park of Rutgers and explants from young and old leaves and from floral buds gave similar results.

Thousands of *Artemisia* plants can be propagated from a single stem cutting. This is a very economical way of propagation. In Zimbabwe vegetative reproduction of the *Artemisia* commenced in October 2004 at Domboshaba Research Station with a single plant from which 200 stem cuttings were planted on-station. (Hanyona, 2005).

ICRAF is facilitating the broad propagation of A-3 by teaching thousands of farmers how to cultivate *Artemisia* from stem cuttings. The programme has extended to four districts in Tete Province – Angonia, Moatise, Tsangano and Makanga – located in North Western Mozambique. (Rumley, 2005).

#### 2.2.4 Planting

China uses seed from wild populations, which is broadcast and subsequently thinned. (See picture of the variation in a in Jingxi County planting).

##### Direct drilling

If supplies of seed are freely available, then direct drilling of seed is the most economical way of plant establishment provided the length of growing season and other environmental factors are suitable and weeds can be controlled. Field experiments have shown that the yield of artemisinin after direct drilling is similar to the yields of transplanting.

Direct drilling of *A. annua* seed can be carried out by various methods ranging from the basic hand application or simple hand pushed single row seeders to sophisticated multi row seed and fertilizer drills. Because the seed are so small it needs to be diluted either by some inert material or by an appropriate neutral fertilizer. In Tasmanian experiments the technique of mixing a 50:50 blend of fine ground limestone and superphosphate with the seed gave successful establishment. The seed should only be mixed in the blend immediately before drilling.

Depth of sowing is critical with a small seeded crop such as *A. annua*. In Tasmanian studies, a depth of drilling of 5 mm gave good emergence and establishment. It is important, with a shallow drilling depth such as this, that the soil be cultivated down to a fine tilth to avoid seeds being sown on the surface. (Wright, 2002).

##### Time of establishment

The choice of time of establishment must allow a sufficient period of rapid growth and the production of vigorous vegetative framework before the commencement of flowering.

The timing will be related to the availability of irrigation water/rainfall, temperature and day-length and to the desired period during of harvest. These factors will vary from region to region but the timing should allow for a seed germination time of about 4 weeks, a further period of about 5 weeks before transplanting and about another 12 weeks to reach maximum dry leaf weight and artemisinin content.

In cool temperate continental climates good establishment and yields have been obtained from both late spring and early summer sowing. In cool temperate maritime climates good results have been obtained from both winter and spring sowing.

Rain-fed crops should be drilled 3 weeks before the onset of the rainy season. E.g. in Kenya, should rains be expected mid-April, then sowing should be done in the last week of February. Water stress as well as low temperatures will accelerate flowering.

The flower inductive photoperiod ranges between 11 and 13 hours for Vietnam genotype and 13 and 15 hours for Chinese genotype. The Vietnam genotype plants flourish about 4-5 weeks



after submitted to the inductive photoperiod, while the Chinese genotype plants flourish in 2 weeks. (Wright, 2002).

### Nursery planting

In Kenya, direct drilling is not used due to the tiny seed (1000 seed weight = 0.03g) and the need for a vigorous seedling. (Stress will induce early flowering). It is recommended to transplant seedlings that have been raised either in trays or in seedbeds and that have produced vigorous, upright plants for transplanting.

EABL plants pelleted F1 and F2 seeds and seedbed seedlings are transplanted to planterflats as a vigorous root system is needed at transplanting. East Africa has a second planting in August. A. annua planted over 2500 m in E. Africa is exhibiting the characteristics of a bi-annual plant.

### Nursery Preparation

The nursery must be sited in a different position every year, to prevent build-up of soil-borne pests and diseases, and to prevent nutrient build up or depletion. Soil fumigation may be necessary prior to planting. Ideally, soil pH should be between 5.5 and 6.5 - the pH should be determined by soil analysis and any lime requirement (in case of acidic soil) should be applied at least 2 months prior to sowing. The site chosen should either be partially shaded, or out of direct sunlight. There should be provision made for using shade cloth or a light mulch over the beds and the nursery should be close to a source of good quality water. Free draining soil is essential to the production of quality plants. Nursery areas should be prepared well in advance of sowing.

The nutrition status of the seedbed should be determined by soil analysis from a sample taken at least 2 months prior to sowing. Correct the nutrition status of the seedbeds according to the results of the soil analysis - do not guess.

Weed control around the site to a distance of 10 meters is advisable and the site should be fenced to prevent access by unauthorized persons or animals. If perennial weeds such as docks or grass species are present, these should be sprayed with glyphosate before cultivation. (EABL).

The area should be cultivated as early as possible, to a depth of 35-40 cm, and raked to a fine tilth, and any weed roots removed. The use of a jembe handle can be helpful in breaking soil clods to the required tilth. A suggested bed size is 1.5 m wide and 20 m long, raised 20 cm high. If the seedlings are to be lifted using a mechanical knife, then the bed width should be just less than the axle length of the tractor. Prior to sowing, the beds should be thoroughly irrigated, to allow residual weed seeds to germinate. It is recommended by EABL that these are then sprayed off with Glyphosate. Proper preparation for weed control in the seedbed is essential- as Artemisia seeds are so small, it can be difficult to identify weed seedlings from Artemisia seedlings at an early stage. (EABL, 2005).

### Protection from the elements

To provide optimum germination conditions, it is recommended that a protective layer be used to prevent dehydration of the seeds after initial imbibing of water and to give an even germination and therefore more uniform plants to transplant.

It is recommended that 60% shade netting be laid over the bed and pegged down. This will help in moisture conservation during the first few days of germination, whilst providing light to the seeds. Once the seeds have germinated, the net can be elevated above the bed to provide shade, without interfering with seedling growth. Alternatively, a light mulch of Rhodes grass hay can be used. It is essential that the stems be well beaten beforehand to ensure no seed enters the seedbed! (EABL, 2005).

#### Sowing in nursery beds

The seed beds should be brought to field capacity the day before sowing, to the full depth of the cultivated soil. There are several suggested methods of sowing. Pelleted seed can be mixed with fine grade sand, which is then sprinkled lightly over the surface of the bed, at the rate of about 1 gm seed per m<sup>2</sup>. Establishing in rows 10 cm apart at the same seed rate will make uprooting the seedlings easier at transplanting, and if this can be done accurately then it is strongly recommended. Note that germination rate will affect sowing density; the germination capacity for the appropriate seed lot should be controlled beforehand.

For raw seed, the seed can be sown like tobacco, using two connected watering cans. The watering cans are connected to each other via a hose pipe with holes at intervals of 10 cm, which will allow the seed to be sown in rows. The seed is added to the water, and the water is agitated to keep the seed in suspension. Two operators walk the length of the bed, pouring the contents on the bed. This method will allow many rows to be sown at once, but does need practice to ensure the whole size matches the walking speed.

The aim should be to produce 500 healthy seedlings per m<sup>2</sup>. EABL recommends that growers estimate an establishment rate of 50%. Thus 1000 seeds should be sown per per m<sup>2</sup>. These can then be thinned out to the correct density. Note that 1 gram of raw seed is sufficient to sow 13 m<sup>2</sup>. Artemisia requires light and uniformly high levels of moisture, at temperatures of 18-20oC for germination. It is essential that the seeds are not covered with soil after sowing - this is one of the main reasons for failures in the nursery.

The plantlets will emerge in 6 or 7 days and as they appear, the cover can be removed, and the plantlets kept moist with regular applications of fine-droplet water from a can or (preferably) a mist-sprinkler. If no shade is used it is essential that the seedbed be maintained at 90-95% of field capacity and this can be done with LIGHT but frequent watering with a watering can.

Seedlings are tiny (the pin is 1 mm wide). (See picture of tiny seeds). This picture is of germinating seeds of one of the 3 hybrid lines ('2/39 x IV') developed by CPQBA in Brazil in collaboration with Mediplant, Switzerland. It was taken one week after sowing.

EABL strongly advises use of shade as this reduces the risk of failure, by keeping the seedbed at an adequate moisture level. Beds will lose more water on a hot, windy day than on a cool, still day. More water will also be lost as the plants become bigger. Initially, the seeds may need watering 3 times per day during the first 10-12 days of germination. The irrigation can then be reduced to once per day, for the next 10 days, and then reduced further, depending on weather conditions. Reducing irrigation will encourage root growth, and the formation of a strong plant. These are guidelines only - it is essential to watch the weather! (EABL, 2005).

#### Sowing in nursery trays

Germination medium for nursery trays: Mix coarse sand, fine compost, good clean top soil and water. Prepare the mixture in a bucket or on a sheet of canvas.

Only quality topsoil, with pH between 6-7, should be used in the nursery trays. If it is necessary to adjust the pH with lime (in the case of acid soil) this should be done at least 2 months before the final preparation of the planting medium.

The soil has to be a free draining, friable, preferably loamy, of intermediate texture, otherwise with a sand content of <60%, and clay content of <40%, such as fine sandy loam and clay loam. Too sandy or clayish soils are not appropriate.

Soils with very high organic matter should be avoided. If a soil contains too many roots and other organic particles it should be sifted, since these particles could contain pathogens which may attack the plants.

Before use the soil must be sifted to remove weeds, debris, stones, sticks and large clods (>1 cm in diameter). The soil must be firm enough to develop a firm clod when the seedlings are removed from the tray at transplanting.

Do not use sand, clay, peat, un-decomposed organic matter, contaminated soil, compacted soil, or soil that has been exposed to high temperatures to fill the nursery bags. Excessively sandy soils will make the clod fall apart, and in clay soils the seedling will not develop a healthy root system.

Heat and boil the germination medium for at least 5 minutes in order to sterilize it. When the mixture has boiled, scoop it out of the still hot water (it may take some patience because it tends to run away). Then spread it on a canvas to cool and drain the excess water. Add a low quantity of a complete fertilizer and some Styrofoam, either as small balls or from boxes that has been carefully broken down into little fragments.

Nursery trays: Trays should be made of water resistant well seasoned hard wood to ensure lasting trays. To improve resistance some lime should be rubbed on the wood. Drill wholes for every 10 cm in the bottom of the tray to insure drainage. Netted lids are made to fit the top of the trays in order to protect the seedlings from animals and fallen leaves.

Store the already prepared germination medium in semi-pervious bags and wait until the medium has the right moisture content (should be moist but not wet). It is also possible to fill the trays directly but during the drying process the mixture may become hard, in which case it has to be reworked and softened. Two hours before sowing the soil in the trays must be watered very well.

Seed sowing and germination: The very small seeds can be sown with the help of a sieve or pelleted seed can be mixed with fine grade sand and then sprinkled lightly on the surface of the medium. After sowing they should immediately be sprayed with water.

The aim should be to produce about 500 healthy seedlings per m<sup>2</sup> or about 250 seedlings per tray. Note that less than 0.1 of raw seed is sufficient to seed one m<sup>2</sup> and 0.04 g is enough to seed one tray.

Seeding should be done in rows to facilitate possible thinning of seedlings and to make it easier to prickle seedlings at the time of transplanting.

It is essential that the seeds are not covered with soil after sowing – this is one of the main reasons for germination failures. The trays should be placed on a bench free of the ground in a

well ventilated site under light shade and protected against wind. Temperatures should be between 18-20°C. Keep the trays covered with a sheet of plastic until the first seeds have germinated.

Three days after sowing the seed will start to germinate and after 6 -7 days all viable seeds should have germinated. The seedlings and the soil must be kept moist but not wet at all times, the seedlings may never dry out. Sprinkle or spray with water every morning and afternoon. Seedlings should be thinned to about 250 per tray.

After 3-4 weeks when the seedlings have reached a height of about 2-3 cm (4-5 true leaves) they are ready to be pricked out and transplanting into pots, cellular trays or an other container system.

Transplanting from nursery trays to pots: Use big plastic pots (diameter about 30 cm) or cellular trays. Fill pots or trays with the same soil medium as described under “Germination medium for nursery trays” and water well.

Two hours before lifting the seedling from the trays, the soil in the trays should be saturated with water. Due to the small size of seedlings (3-4 cm), transplanting has to be done extremely carefully. Use a small rounded knife to lift the seedlings and be careful not to damage the roots.

Make wholes in the pot medium big enough to allow the seedlings to be planted with their entire, unbend root systems. The base of the stalk must not be covered by soil. Transplant 6-7 healthy seedlings to each pot or one into each cell in the trays. Place the pots in half shade and water regularly morning and afternoon.

About 8 weeks (10 true leaves) after sowing (4 weeks after 1st transplanting) the seedlings should be ready for hardening off. To harden the plants, gradually maximise the sunlight, while reducing the watering to a minimum. The process must not be excessive to avoid stress-induced reactions causing early flowering. Do not fertilize the plants at this time.

Seedlings should be ready for transplanting to the field about 11 weeks after sowing (5 weeks after 1st transplanting), when they are about 10-12 cm tall.

Two hours before lifting the seedling from the pots, the soil in the pots should be saturated with water.

#### Diseases in the nursery

It is possible that diseases can have a serious impact on seedling survival and growth. If seeds are sown in a manner that will give rise to very high populations of plants, or if they are over irrigated or over fertilized then they may be susceptible to damping off. This condition is a combination of a number of fungi and will cause serious losses if not treated. The local contractor (in Kenya EABL) must be informed before any treatment is made in order to obtain recommendations on what to use.

Damping off can occur as soon as the radicle emerges from the seed or later in the emerging or growing plant. After emergence symptoms are a dark coloured water-soaked lesion at or near the soil surface. The young stem is constricted by the attack and becomes soft; the plant eventually falls over and dies. Older plants may be attacked under certain circumstances, but

the plant develops resistance as it gets older and although it may survive, its vigour will be limited in the field. (EABL, 2005).

#### Seedling fertilisation

It is important not to over fertilize in the nursery as this will lead to plants that are out of balance with the ratio of root/top growth incorrect. Having well grown seedlings to transplant is essential for plant survival and therefore yield. Use of natural manures and composts is recommended, and there should then be no need for additional artificial fertilisers. If well rotted manure is not available, EABL recommends the use of DAP at no more than 10 gm per m<sup>2</sup> (one level teaspoon). If the plants look yellow, urea mixed with water can be added- do not exceed 10 g in 10 litres of water.

Compound fertiliser can also be used; use a compound that has as low nitrogen as possible, and a higher phosphate. Regardless of fertiliser, do not exceed 10 g per m<sup>2</sup>. (EABL, 2005).

#### Seedling development

Seedlings will begin to show the typically serrated pattern of the leaves at the first true, 2-leaf stage. Any weeding is safe after this, if done too early it will disturb the roots and either cause plant losses or will delay the development of the plant and give uneven plant sizes at transplanting. The plants will then display a rosette pattern, and will start to elongate after this. They are ready to transplant approximately 7 weeks after sowing, at about 10-12 cm high.

Some 6 weeks after the seeds were sowed; the plantlets will be ready for hardening off, and then a week later they will be ready for transplanting. To harden the plants, maximize the sunlight, and reduce the watering to a minimum. This is a critical stage of the plant's development and the hardening process must not be excessive, or it will result in a stress-induced reaction that may cause the plant to flower soon after planting with a resultant loss of yield. Fully remove the shade cloth if this was used and reduce the watering to stress but not wilt the plant. Do not fertilize the plant at this time. (EABL, 2005).

#### Nursery practice

The seed beds should be kept completely free from weeds, this will give good even plants free from diseases and with straight stems. The plants should be 10-12 cm high when transplanted. To be able to lift the plants successfully, the beds should be well watered the evening before transplanting is to start. This watering must be very thorough and the beds should not be able to absorb any more water, with the soil being wet to at least 50 cm deep. This will ensure that the soil in the immediate root zone contains adequate moisture during transplant.

The next morning, to lift the plants, use a fork or a shovel and GENTLY raise the soil being careful not to damage the plant roots. It is important to have as many roots as possible to support the plant after it is put in the field and the seedlings should be "teased" from the soil to prevent damage. Only lift as many seedlings as you can plant in an hour. The lifting operation can also be done mechanically, using a tractor-towed knife.

#### Transporting the seedlings

Ideally the transplanting should take place on a cloudy or rainy day. The seedlings should be carried to the field in a box that is lined with wet hessian and covered with wet cloth or hessian bags to stop the plants drying out. The shorter the time the plant is out of the ground, the quicker the recovery. (EABL, 2005).

## Field planting

Influence of Planting Date and Harvest Time: Seedlings of *A. annua* were transplanted into a central Indiana, United States field on April 27, May 17, June 10, and July 13, 1987, and plant samples (for growth and essential oil) were obtained every two weeks until the first frost. The May transplanting date produced the highest fresh yields and tallest plants, while the May and June plantings had the highest percentage of essential oil. Regardless of planting date, all plants began to flower by mid-August, with maximum concentration of essential oil produced in mid-September (peak flowering stage). Results from the 1988 growing season (data not presented) were similar to those obtained in 1987. (Simon et al., 1990).

It is preferable to plant after the rains have started which will mean that the soil has high moisture content. Holes or trenches should be made in the ground deep enough to hold all the roots vertically. It is important not to have a bend in the roots, if this happens the plant has greater risk of failing due to poor support and then blowing over. It may also have a poor root system and be unable to survive dry periods or not be able to get sufficient moisture that will result in early flowering, thereby reducing the amount of crop produced.

Ideally there should be a team of people at planting, one to make the holes, one to transport the seedlings and give them to the planter and the person who plants the seedling, and covers the plant after. A much straighter planting can be achieved if the planters are able to follow a tine line, or a marked string. Seedlings that are taller or older than the ideal transplant age can be planted a little deeper in the soil without any ill effects. This will help minimise lodging and weak stems later in crop life.

It is recommended that a small amount of the product called Kicstart® be added to the irrigation water given to each plant at transplant, to minimise transplant shock. In trials completed in 2001 the use of this product gave a 30% increase in height of *Artemisia* 2 months after planting compared to those plants that received no Kicstart®.

Together with this, EABL recommends the use of a pyrethroid or natural pyrethrum (such as Flower DS®) to control cutworm, which can attack the young transplants. It is essential to wear correct clothing, and to follow the manufacturer's instructions. Be sure to keep spare seedlings on hand for up to two weeks after planting to enable gap-filling should some plants be lost to cutworm.

The soil around the plant roots must be pressed firmly into contact with the plant - this will prevent the plant from wilting too much and increase the rate at which it recovers from the shock of transplanting. (EABL, 2005).

## Planting density

If inter-row cultivation is used to control weeds before the rows close then inter-row and intra-row spacing of 0.5-1.0 m (1-7 plants/m<sup>2</sup>) are appropriate. If effective herbicides are available, then yields per unit area can be increased by using higher planting density. Planting densities of 3, 7 and 11 plants/m<sup>2</sup> was tested and the highest biomass was obtained at the highest planting density. (Simon et al., 1990). In Tasmania, planting densities of 1, 5, 10, 15 and 20 plants/m<sup>2</sup> was tested. It was found that leaf dry matter yield increased up to a density of 20 plants/m<sup>2</sup>. However, yield at 10 plants/m<sup>2</sup> was about 90% of the maximum of 6.8 t/ha. (Laughlin, 1993).

There are a number of trials that have been conducted in Kenya to determine the best plant number per hectare. Based on several years experience, (using vigorous seedlings) the current recommendation is to plant at a spacing of 1.0 m between plants and 1.0 along the row. This will give a plant population of 10,000 per hectare. For F2 plants, the spacing can be reduced to 1 m x 0.75m, to accommodate for the decreased yield.

Measure the distance between plants, do not guess. If there is just 5 cm too much between plants it will reduce the plant numbers by almost 500 per hectare and production will be correspondingly lower. Use a marked measuring stick. (EABL, 2005).

The response of *A. annua* to plant spacing and nitrogen fertilization was evaluated in 1985 and 1986 with three populations established from transplants: high density, 30 cm x 30 cm (111,111 plants/ha); intermediate density, 30 cm x 60 cm (55,555 plants/ha); and low density, 60 cm x 60 cm (27,778 plants/ha). Plants in each spacing received three levels of nitrogen fertilization (0, 67, and 134 kg N/ha) applied as a preplant broadcast application.

Plants from the densely populated treatment (111,111 plants/ha) produced an average fresh weight of 275 g/plant, as opposed to 430 g/plant from the intermediate and 750 g/plant from the lowest populations. However, total biomass (fresh weight kg/ha), was greatest from the higher density population (Table). Plants of the most densely populated treatments were slightly taller, produced less side shoots, and had longer internodes with little lateral growth than the lower densities. Number of side shoots per main stems decreased as density increased. While yield increased with added nitrogen, the greatest growth (herbage and essential oil content) was obtained with 67 kg N/ha (Table).

Increasing density tended to increase essential oil production on an area basis, but highest essential oil yields (85 kg oil/ha) was achieved by the intermediate density at 55,555 plants/ha receiving 67 kg N/ha (Table). Artemisinin content was not analyzed in this study because the instrumentation and method of analysis had not yet been developed in our laboratory. (Simon et al., 1990).

### 2.2.5 Water

The plant is able to withstand dry conditions when it is fully established, but stress at early point in its life can induce premature flowering or leaf atrophy, and this in turn may be reflected in a reduced artemisinin content in the leaf and a lowering of total dry matter production per hectare which results in lower returns for the crop.

A rain-fed crop requires between 600 mm and 650 mm of rain during the season, so timely planting is important in all circumstances. Where supplementary irrigation is available this may materially assist in plant survival at establishment and the production of larger amounts of dry matter per hectare. The most critical stage of growth for additional water is in the first 12 weeks of the crop life after transplanting. Each property will have different water requirements that will be influenced by soil type and climatic patterns and it is not possible to generalize on the timing of supplementary irrigation. This should be discussed with the local contractor (in Kenya - EABL) who will advise on the need for water.

The general principles of irrigation applying to deep-rooted crops such as Lucerne also apply to *Artemisia*. They are:

- 1) Water as little as possible but apply at least the equivalent of 30 mm rainfall per irrigation once the plants have established. This will encourage the roots to seek water and assist the plant to withstand short periods of water stress should this occur.
- 2) Adjust the frequency of irrigation to take into account the surface area of the plant and the growing conditions. The plant will transpire greater volumes of water as it becomes larger and will also do so in periods of hot, windy weather.
- 3) Do not apply more water than necessary, as it will result in leaching of nutrient, which is environmentally unacceptable and is wasteful of resources and also may deprive other potential users of the opportunity to irrigate.

### 2.2.6 Fertility

There are few specific experimental data on field response of *A. annua* to phosphorus and potassium. Trials in USA (Indiana) compared different rates of nitrogen fertilizer supplying 0, 67 and 135 kg N/ha and obtained the highest total plant yield with 67 kg N/ha. (Simon et al., 1990). In India, sand culture studies with an American strain of *A. annua* also showed that nitrogen deficiency was associated with a large decrease in artemisinin.

Nitrogen is a very mobile element and can easily be leached out of the root zone especially in regions of high or concentrated rainfall. In these areas the method and timing of nitrogen fertilizer application is very important. Banding of nitrogen near the seed or plant rows, 2-3 split applications or slow release nitrogen may be ways of reducing leaching. (Wright, 2002).

In Kenya, many soils are generally low in Phosphate and Nitrogen and some also have a low pH. The addition of fertilizers should be based on the results of a soils test. If this has not been done then it may be necessary to add fertilizers based on previous experience on the property or in the district.

The type of fertilizer used is important; trials done by EABL suggest that the use of DAP may result in leaching of nutrients under flood irrigation and for this reason DAP is not recommended as it is environmentally unsatisfactory. Another commonly used fertilizer in Kenya is Sulphate of Ammonia, however this lowers the pH of the soil and should only be used in areas with a high pH. If used in soils with a pH less than 6.5 it may reduce the availability of micronutrients that have been shown to be important in the production of artemisinin, the active component in the crop.

Phosphate should be applied evenly before the last cultivation and worked into the soil to a depth of 12-15 cm (approximately 6 inches). Trials are currently being conducted to find the optimal rate and time of nitrogen application, which will result in as much leaf as possible. Currently, EABL recommends a 50-50 split of the Nitrogen application, 50% at planting and 50% when the crop reaches 50 cm tall.

A well-balanced foliar fertilizer containing micronutrients should be sprayed twice during the crop's growing period. The first should be at 4 weeks after transplanting and the second 4 weeks later. This will assist in the formation of artemisinin and give higher yields per hectare. The product may vary from farm-to-farm according to the soil type and should be determined by a soils analysis. EABL is actively investigating foliar feeds to establish which foliar feeds produce the most branches, and which assist in improving artemisinin content. (EABL, 2005).

### 2.2.7 Weeding



Artemisia grows slowly initially (whilst it develops its root system) and may take up to 12 weeks to cover the surface of the ground. Careful weeding is required to ensure that the plants are not inhibited in their growth and unless this is conducted often enough to prevent serious competition the yields be lower than anticipated. Excessive weed growth can also pre-dispose crops to disease and in a wet season this can cause losses that would not otherwise occur. Weeds also harbour pests, and cutworm and moles will be a greater problem in weed-infested areas.

Row spacing must be adjusted to type of weed control practiced. Whether hand control of weeds, inter-row cultivation by hand pushed or tractor drawn implements are used careful thought must be given to row spacing to allow easy access while the crop is small and before the rows close.

If the crop is to be drilled, weed control in the early stages of growth are more critical than for transplants. The young seedlings are very small and can easily be choked by weeds. In this situation weed control with herbicides are the most efficient method. (Wright, 2002).

Arteether, a derivate of artemisinin, has been shown to be a very effective growth inhibitor of dicotyledonous weeds (Bagchi et al., 1997). Application of 2.2 kg active ingredient a.i./ha napropamide before transplanting gave good weed control without phytotoxicity in the USA (Simon and Cebert, 1988); chloramben was very effective when applied at 2.2 kg a.i./ha before emergence; trifluralin at 0.6 a.i./ha incorporated before transplanting followed by fluazifol at 0.2 + 0.2 kg a.i./ha broadcast after emergence and acifluorfen at 0.6 kg a.i./ha after emergence. (Bryson and Croom, 1991).

Weeds can be a concern in Artemisia crops and are the focus of considerable trial work to establish the use of herbicides. In East Africa, no herbicide has yet been registered for use on Artemisia, although these trials will soon be placed. Response to some herbicides has not been consistent in trials conducted on varying soil types and until crop safety has been established by further trial work no herbicides can be recommended for the crop. EABL does have experience in use of specific herbicides in some regions. Although it has been shown to be effective in Tanzania, please note that Fusilade has not been registered in Kenya for use in Artemisia, and EABL cannot condone the use of this chemical. (EABL, 2005).

Mulching is essential to retain soil moisture and suppress weeds.

## 2.2.8 Harvest

### Time of harvest

The optimum time of harvest will depend on the target compound desired and on the variety grown. If artemisinin is the main objective, then maximum yield, in most cases, occurs at early bloom, though studies of Vietnamese selections have given maximum yield at the late vegetative stage. If oil or a combination of oil and artemisinic acid is the main objective then full bloom would be the best time of harvest. (Wright, 2002).

Harvest timing is critical, as artemisinin content tends to climb steeply during late active growth, then to plateau briefly and finally, to fall off sharply once flowering has initiated, with its corresponding leaf drop. The plant begins to flower from laterals originating at the bottom of the main stem. With the current variety (East Africa), when approximately half to three quarters of the plants show signs of bud initiation, artemisinin content will be at a maximum and the plants will be ready to harvest. (EABL, 2005).

An important factor for harvest is optimal leaf artemisinin content as 'before flowering' is not sufficient. EABL are using the 'Iatrascan' technique which needs a nearby laboratory and takes 2 hours. In Vietnam, an 'in the field' technique which takes 10 minutes has been developed and results in fast farmer payment; probably a chromatographic strip technique - Nguyen van Thuan (Vietnam Research Centre of Medicinal Plants) will provide details for this record.

#### Location of Natural Products

Essential oils and artemisinin were assumed to be associated with secretory cells based on the association of mono- and sesquiterpenes with well-defined secretory structures. (Croteau, 1986 and Henderson et al. 1970). Relative distribution of artemisinin see Table. Leaves had 89% of the total artemisinin in the plant with the uppermost foliar portion of the plant (top 1/3 of growth at maturity) containing almost double that of the lower leaves. (Charles et al., 1990). Kelsey and Shafizadeh, 1980 reported that 35% of the mature leaf surface is covered with capitate glands which contain most of the monoterpenes and virtually all of the sesquiterpene lactones. Essential oils from *A. annua* are similarly distributed, with 36% of the total from the upper third of the foliage, 47% from the middle third, and 17% from the lower third, with only trace amounts in the main stem side shoots, and roots. (Simon et al., 1990).

#### Method of harvest

EABL are developing a novel economic technique of mechanically harvesting the top third of the plant when green and irrigating for re-growth. (See also - Laughlin, 1995). (EABL, 2005).

Plants can be cut at the base using a sharp panga. They are then laid in a line or stoked to dry in the field but they should not be in a pile as they will not dry evenly and considerable leaf loss may occur through rotting in the centre of the pile. It is essential to ensure that the wind can penetrate the rows of plants to assist drying and the plants should be placed in such a way that this can occur. The plants will dry sufficiently in 5-10 days depending on the weather and after this the leaf can be removed from the branches (at a moisture content of approximately 30-50%), and taken for drying under protection. This is essential as the stems and branches have little of the artemisinin in them and must be removed before dispatch to the store. The leaf will eventually dry crisp each afternoon and then each night will absorb water from the air and become limp in the early morning, (due to its hygroscopic nature). While it is limp it will not fall off the plant and this is the time to move it from the field. Experience has shown that this should not be done after 10 in the morning to avoid the leaf falling from the plant and being lost.

A harvesting technique including a first cut with a mower 10 cm above ground level, 10 days wilting and drying in the field and a final pick up with a forage harvester and trailing bin has been tried in Tasmania. The method has been given a very preliminary screening and although feasible it involves the problems of sorting leaves and flower from the stems and of drying the final harvest product. (Wright, 2002).

#### Leaf and flower removal

Leaves (and flowers) can be removed from the branches in several ways. Placing the dried plants on a large piece of hessian or tarpaulin and driving over it with a tractor. Manual removal of the leaf is also very easy, albeit time-consuming, and can be done over a tarpaulin to prevent loss of leaf.

If a tractor is used, the leaf must then be sieved to remove the twigs and branches and this should be done first with 5 mm mesh (coffee wire), then with a 3 mm sieve and finally some hand sorting which will give a good sample. There should be no more than 5% content of plant parts other than leaf in the product dispatched to the store. Sieving should not be necessary if the leaf is removed by hand, during which the leaf can simply be stripped off the branches with minimal effort.

#### Leaf drying

Dried leaf should have a moisture content of no more than 12% and this is easily attainable with sun-drying. EABL is investing in some mobile tunnels and sieve tables in which the leaf can be dried faster, out of risk of rain and wind damage. (EABL, 2005).

The wilting of *A. annua* in the field after harvesting with exposure to direct sun has in some cases reduced the artemisinin content. This has occurred in India and Oregon, United States. In Oregon, samples were dried (1) in open sun, (2) in the sun but protected by paper bags and (3) air dried under cover at ambient temperature. Air drying under cover gave the highest artemisinin content and direct sun drying the lowest. In this study the maximum air temperature was 30°C and the maximum sample temperatures were: sun 42.2°C, shaded 22.8°C and bagged 35.6°C. (Wright, 2002).

Two field experiments were carried out in cool temperate maritime latitudes in N.W. Tasmania (41oS) to assess whether wilting and drying *A. annua* plants in the field after harvest had any detrimental effects on artemisinin or its precursor artemisinic acid. In the first two experiments whole plants were cut off at the base and left in situ for 1, 3 and 7 days (experiment 1) and for 7, 14 and 21 days (experiment 2). The maximum air temperature in this trial was 22°C. Expt. 2 included two additional treatments: (i) shade drying whole plants under ambient conditions in the field for 21 days and (ii) drying leaves, detached at harvest, for 21 days under ambient conditions inside in the dark. The effects of all of these treatments were compared with oven drying (35oC) leaves which had been detached immediately after harvest. Field drying for 1, 3 or 7 days had no adverse effect on either artemisinin or artemisinic acid in Expt. 1 and all leaf concentrations were similar to oven drying. Field drying for 7 days in Expt. 2 also gave artemisinin and artemisinic acid levels similar to oven drying. However there was a trend for sun-, shade- and dark drying for 21 days to give higher artemisinin than oven drying although artemisinic acid was unaffected. Field drying may be a way of reducing the cost of anti-malarial drugs and the dual production of oil and artemisinic acid is a possibility. (Laughlin, 2002).

#### Threshing area

The threshing area must be a separate zone free from rodent entry and other possible sources of contamination. Domestic animals and pets are not permitted on this site and all due diligence must be exercised to ensure that a clean product is created when threshing the plant material. (EABL, 2005).

The leaf represents approximately 20-25% of the biomass of the plant and there is a large amount of residue remaining after threshing - between 12 and 20 tons of dry stem and stalk per hectare. This can be returned to the field as organic matter and as it also burns well can be used as fuel in kuni boosters. Trials are being conducted to evaluate the use of the stems as supports in the horticulture industry, which can bring in an excellent additional financial return per hectare. The pungent smell of the stem may have a role in repelling insect pests in horticultural crops.

## Artemisinin content

Wild harvesting of *A. annua* has the disadvantages of sparse distribution, low artemisinin content and unstable yields, which increase the production and processing cost. Usually, only an artemisinin content over 0.6% has commercial value.

Most collections of artemisia derive from natural stands with highly variable artemisinin content, some as low of 0.01%. Selections from Chinese origin vary from 0.05 to 0.21%. (Janick, 1995).

## 2.3 Improvement

### 2.3.1 Genetic resources

Various specialty herb seed sources sell *A. annua* seed for the wreath market. (See S. Facciola, 1990. *Cornucopia: A source book of edible plants* Kampong Publications. Vista, California).

Indiana Center for New Crops. Horticulture Bldg 1165, Purdue University, West Lafayette, IN 47906-1165 has small amounts of seed for distribution. (Janick, 1995).

Harvesting wild *A. annua* before the flowering stage for high artemisinin content, as it takes place in China, results in depletion of the seed and is a threat to the germplasm resources. Therefore, priority should be put on investigation, characterization and collection of *A. annua* germplasm. This could be through the CAAS/IPGRI Centre of Excellence for Agrobiodiversity research and Development, which has a branch in Yunnan, China.

### 2.3.2 Varieties

The genetic base is restricted and needs to be broadened.

The main African seed source is Mediplant (Switzerland) expensive F1 hybrid 'artemis' and resulting F2 populations are used. (Delabays et al., 2001).

Brazil (CPQBA-UNICAMP) has developed 3 hybrid lines in collaboration with Mediplant, Switzerland; 2/39 x IV, 1V x 2/39, and Ch x Viet.55. (Magalhães et al., 2004a and 2004b).

An improved agro-technology of high yielding *A. annua* variety 'Jeevan Raksha' (with artemisinin yield up to 1% content) has been developed at CIMAP, Lucknow and transferred to an industry (M/s IPCA Laboratories, Mumbai). The industry has already started a small area of cultivation of *A. annua* cv. 'Jeevan Raksha' in Uttaranchal state. Also cv. 'CIM-Arogya' having up to 1.2% artemisinin content has since been developed. 'CIM-Arogya' under north Indian condition yields 4500-4800 kg of dried herb per ha and up to 25-30 kg/ha of artemisinin can be extracted from this cv.

Mert et al., 2002 have developed three ecotypes ('Adana', 'Samankaya' and 'Serinyol') in Turkey.

CIMAP has recently developed a new variety of *A. annua* "Jeevanraksha" containing high levels of artemisinin. (Tandon et al., 2003).

Three mutation lines are being field tested by the National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand.

The anamed (Action for Natural Medicine) coordination in Germany has committed itself to making hybrid seeds available; one particular hybrid, named "Artemisia annua anamed", or "A3" is available.

### 2.3.3 Breeding and biotechnology

CIMAP, Lucknow is using molecular breeding techniques with *Agrobacterium tumefaciens* to enhance the production of artemisinin. An *A. tumefaciens*-mediated system of high efficiency of genetic transformation and regeneration of *A. annua* was established by Han et al., 2004.

The University of California, Berkeley is in the process of identifying a few more genes in *A. annua* that, if transplanted into *Escherichia coli* could enable the bacterium to go a few extra steps in the chemical process and actually produce artemisinic acid. A ninth enzyme was added that directed Professor Jay Keasling's team down the path toward artemisinin, and they now expect that three more enzymes will be needed. Once they have the artemisinic acid needed they will be ready for industrial production of the drug. (Pescovitz, 2005).

The enzymatic route for artemisinin in the plant was elucidated by Dafra Pharma and Plant Research International and one of the key enzymes, amorpha-1,4-diene, was isolated. Further research has resulted in valuable additional information on the enzymatic steps leading to the biosynthesis of artemisinin by combining microbial fermentation and chemistry. (Mincke and Toussaint, 2005).

## 2.4 Products and uses

### 2.4.1 Processing

After threshing and sieving, the leaf must be placed into polypropylene bags supplied by the local contractor (in Kenya EABL), thoroughly sealed to prevent insect or rodent entry and labelled with the Bag Labels provided. These labels must be completed accurately as they are an essential part of the traceability process. Once labelled, the bags must be placed on pallets in a clean, dry store to await transportation. Only bags that meet the conditions as set out in the contract may be used. The local contractor (in Kenya EABL) must reserve the right to reject crop sent in other such packaging. Care should be taken to ensure that stored leaf does not become too hot, as this can have a detrimental effect on Artemisinin content. (EABL, 2005).

In a number of situations the extraction of artemisinin or artemisinic acid may take place at a considerable distance from the site of the *A. annua* production. In these cases the volume and weight of the plant material may be a problem. A crude extract with an organic solvent such as hexane is a possible solution to this problem in that the volume of the extract would only be 5-10% of the original plant material. (Wright, 2002).

The extraction and purification technology (see diagram) of artemisinin using the hexane method was described by de Vries et al., 1999 in their book "Production and Application of Artemisinin in Vietnam". See the methodology described.

Recovering artemisinin from *A. annua* by high-pressure extraction with CO<sub>2</sub> at 60-85 bar and 20-50°C, is a new technique. The recovery comprises: (a) grinding the above ground parts of the plant to powder; (b) subjecting this to high-pressure extraction with CO<sub>2</sub> for recovery of artemisinin; (c) isolating artemisinin from the purified extract mixture; and (d) preparing, from artemisinin, a solution for use in preparation of liquid dosage forms. (Pulz et al., 2005).

Essential oils of *A. annua* can be extracted via steam distillation in units similar to that used in the commercial distillation of peppermint and spearmint. Essential oils of *A. annua* were extracted in studies via hydrodistillation with a modified cleverger trap (Simon & Quinn, 1988) and chemically characterized by gas chromatography (GC) analysis using a fused silica capillary column (12 m x 0.2 mm id) with a OV 101 (Varian, polydimethylsiloxane) bonded phase. Direct injection of 0.5 ml of essential oil samples with He as a carrier gas (100:1 split vent ratio) and oven temperatures held isothermal at 80°C for 2 min and then programmed to increase at 3°C/min to 210°C gave complete elution of all peaks (sensitivity 10<sup>-10</sup>). The injector and detector temperatures were 210°C and 300°C, respectively. Confirmation of essential oil constituents was based upon comparison of retention time with standards and via GC/Mass Spectroscopy analysis.

#### 2.4.2 Characteristics

Toxicities are unknown but pollen is extremely allergenic. (Duke, 1983). Skin contact with the plant can cause dermatitis or other allergic reactions in some people. Likely to be toxic as are other members of the genus. (Janick, 1995). The taste is bitterly aromatic, strongest when grown in dry, poor soil in full sun.

Essential oils of *A. annua* are comprised of many constituents with the major compounds including (in relative % of total essential oil) alpha-pinene (0.032%), camphene (0.047%), β-pinene (0.882%), myrcene (3.8%), 1,8-cineole (5.5%), artemisia ketone (66.7%), linalool (3.4%), camphor (0.6%), borneol (0.2%), and β-caryophyllene (1.2%). (Simon et al., 1990).

Artemether, artesunin, artemisinin are derivatives or extractions of *A. annua*. The species is different from other members of the *Artemisia* family as it seems to have neuro-toxins at extremely low levels.

Artemisinin is not an alkaloid or an amine as the name suggests. This compound is a sesquiterpene lactone peroxide. The endoperoxide linkage is unusual for an antimalarial compound. Structure activity studies carried out indicated that the presence of the peroxide bridge correlates with, and is essential for, the antimalarial activity.

The primary fragrant component of *A. annua* is the monoterpene artemesia ketone, accompanied mainly by other monoterpenes. To retain these compounds in a decoction, it is recommended that the herb be decocted for no longer than 5 minutes.

Nonvolatile sesquiterpenes can be recovered from the plant by solvent extraction, some of which show high antimalarial activity. There are at least 20 known sesquiterpenes including artemisinin (arteannuin A), arteannuin B, artemisitene, and artemisinin acid. (Janick, 1995).

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A liquid chromatography-mass spectrometry (LC-MS) method with selected ion monitoring (SIM) has been developed and validated for the analysis and standardization of artemisinin in *A. annua*. This method is simple and accurate and requires only an 11 minutes per sample running time for the direct detection and quantification of artemisinin. To accurately analyze artemisinin, SIM is used to record the abundance of the  $[M - 18 + H]^+$  ion peak at  $m/z$  265.3, with a scan range between  $m/z$  250 and 270. Quantification is based on the LC-MS peak area of artemisinin, and the standard curve is used for calculation. (Wang et al., 2005).

#### 2.4.3 Uses

**Food additive:** The plant yields 0.3% essential oil. (Chopra et al. 1986). This has an agreeable, refreshing and slightly balsamic odour and has been used in perfumery and as flavouring in spirits such as vermouth. (Duke, 1983 and Chopra et al. 1986).

**Materials:** Artemisinin also has phytotoxic activity, even in *A. annua*, and is a candidate as a natural herbicide. (Duke et al., 1987 and Chen et al., 1991). The plant is used in China as a medium for growing *Aspergillus* which is used in brewing wine.

The plant is used in China as a medium for growing *Aspergillus* which is used in brewing wine.

Used in the crafting of aromatic wreaths. (Janick, 1995).

**Medicinal:** *A. annua* has long been used for medicinal purposes in China, and was recommended as a treatment for chills and fevers (symptoms of malaria) in 340 AD. The active ingredient, artemisinin (qinghaosu), was first isolated by Chinese scientists in 1972 as part of an antimalarial drug discovery program established in response to a request from North Vietnam during the Vietnam War. (Klayman, 1985 and Honigsbaum, 2001). Artemisinin, an important natural antimalarial effective against multi-drug resistant *Plasmodium* spp. (Janick, 1995).

The leaves and especially flowers are reported to have antiperiodic, antiseptic, digestive, febrifuge, antiprotazoal, antibacterial, and supposedly antiyeast properties. (Yeung. Him-Che, 1985). An infusion of the leaves and flowers is used internally to treat fevers, colds, diarrhoea etc. (Foster & Duke, 1990 and Chevallier, 1996). Externally, the leaves are poulticed onto nose bleeds, boils and abscesses (Foster & Duke, 1990 and Bown, 1995). The leaves are harvested in the summer, before the plant comes into flower, and are dried for later use (Chevallier, 1996).

The plant contains artemisinin, this substance has proved to be a dramatically effective anti-malarial against multi-drug resistant *Plasmodium* spp ([Duke and Ayensu, 1985, Bown, 1995, Chevallier, 1996 and Duke, 1983). Clinical trials have shown it to be 90% effective and more successful than standard drugs. (Chevallier, 1996). In a trial of 2000 patients, all were cured of the disease. (Bown, 1995).

Administration vary from 400-1600 mg/day for 2-7 days, some studies indicate 20 mg/kg body weight/day possibly followed up with quinine 30 mg/kg body weight for 3 days to lower the recurrence rate. A high dose treatments, as well as a sufficient course of therapy, may be essential to avoiding recurrence as well as avoiding development of resistant strains.

In addition to antimalarial effects, artemisinin was found to have promise in treating the parasitic diseases schistosomiasis and clonorchiasis (common in China and Africa, affecting over 200 million people each year) caused by trematodes (blood flukes). Artemether is now being used for prophylaxis against schistosomiasis, in combination therapy with praziquantel it is used to treat the disease. *A. annua* is also included in effective treatments for leptospirosis, a bacterial disease that usually infects humans from animal waste contaminating water supplies.

Artemisinin becomes cytotoxic in the presence of ferrous iron. Since iron influx is high in cancer cells, artemisinin and its analogs selectively kill cancer cells under conditions that increase intracellular iron concentrations. Since it is relatively easy to increase the iron content inside cancer cells *in vivo*, administration of artemisinin-like drugs and intracellular iron-enhancing compounds may be a simple, effective, and economical treatment for cancer. (Singh and Lai, 2001).

Some types of cancer is associated with infectious conditions and there is considerable accord that patients with these types of cancer will not fight the disease until the infections are resolved. This makes *A. annua* interesting because of its antibiotic properties. The inability to run a fever suggests compromised immunity and a metabolic rate that is not efficient enough to throw off infection and this is a pattern common to many cancer patients.

Side effects of artemisinin treatments at normal to high therapeutic doses appear to be rare and mostly involve gastro-intestinal reactions such as nausea, vomiting, and diarrhea (with or without intestinal cramping). In a large study in Thailand comparing high-dose artemisinin derivatives (artemether and artesunate) alone versus in combination with mefloquine, the incidence of adverse effects with the artemisinin compounds was reported to be 34% for loss of appetite, 16% for nausea, 15% for dizziness, and 11% for vomiting. Mefloquine greatly increased the incidence of side effects, doubling the rate. In a clinical trial comparing artesunate injection with chloroquine and with the combination of quinine and resorcin, no adverse effects of artesunate were reported, while dizziness was a common complaint with the drug therapies.

The seeds are used in the treatment of flatulence, indigestion and night sweats. (Foster & Duke, 1990).

Environmental: Ornamental fragrant often grown for fresh and dried arrangements and wreath making (it holds colour and fragrance very well). It is popular in dried arrangements because of its fluffy look, abundant lacy open branches and pleasant fragrance. It also makes a graceful accent in the back of a flowerbed or a pretty quick screen.

## 2.5 Notes

Contacts:

This record is under construction (November 2005) in collaboration with WHO:.....

As well as various Internet sites consulted (see the "Links" icon) information has been supplied personally by Allan Schapira, WHO; James Simon, Rutgers/ASNAPP; James Duke, ARS; Barney Gasston, Africa Bio-Medica Ltd. & EABL; Qizhan Tang, Gaungxi Academy of Agricultural Sciences (GXAAS, China); Nguyen Van Thuan, Vietnam; Anil Singh, CIMAP, India; José Abramo Marchese, Federal University for Technology of Paraná, Brazil; Pedro M.



de Magalhães, UNICAMP/CPQBA, Brazil; John Laughlin Tasmania, Australia; Dan Gudahl, Winrock International (re. Cameroon); Daw Khin Phyu Phyu, Myanmar; to be continued with more contacts and contact data.....

### 2.5.1 Pest notes

The most common pests that have been observed to affect the Artemisia plants are cutworms and black field crickets. Both of these pests cause their damage when the plants are young, and both are more common where the field has been left to get weedy between crops.

Cutworms are the larvae of a moth. The caterpillars burrow into the soil by day and emerge to feed at night. They attack the plants at the point where it touches the soil and eat through the stem, causing the plant to fall over. Often, several plants in a row will be found cut off at ground level. Plants that have not been entirely cut through will look severely wilted, and should also be replaced. The caterpillar is grey-green and curls into a circle when it is found.

Black field crickets are small black jumping insects with large back legs like locusts. In their early stages they may be brown in colour but older insects are black. They are found hiding under big clods of dirt or in cracks in the ground.

Caterpillars and aphids have been observed to attack the plant at later stages, eating the soft stems of the leaves, or sucking from the stem respectively. This is not a cause for concern as the pests die after a few days, and the plant will re-sprout from the injury site. Such situations should be monitored carefully, but no action should be taken without speaking to the local contractor (in Kenya EABL).

Many insects (including beneficials) take refuge in Artemisia. Whiteflies have also been observed in the crop, although no feeding signs have yet been observed. It is doubtful such insects will be able to breed on the crop due to the shape of the leaf.

Few diseases are reported in Artemisia and they are not considered significant after plant establishment. (EABL, 2005).

Members of this genus are rarely if ever troubled by browsing deer.

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### 3.0 Medicinal applications

In this section the pharmacopoeial name is given followed by uses (parts used, preparation, constituents, standards, methodology), pharmacology (systems, ailments, clinical trials, quality control, precautions, toxicology), wild harvesting (methodology, legislation, conservation), marketing, cultivation, notes and a bibliography.

#### 3.1 Pharmacopoeial name

Herba Artemisiae Annuae (The Pharmacopoeia of the People's Republic of China, 2000; English edition).

#### 3.2 Uses

Malaria is a major disease in the developing nations, particularly in Africa. Every 12 seconds, somewhere in the world, somebody dies from malaria and about one to two million deaths are reported annually. Children and pregnant women of poor families are particularly at risk. The disease also increases the risk of transmission and infection by other diseases such as HIV/AIDS.

Over the last decades, there has been a continuing deterioration of the malaria situation in many parts of the world. This is particularly so in areas where *Plasmodium falciparum* has developed resistance to traditional treatments against malaria, such as chloroquine, sulfapyrimethamine combinations and, to some extent, to quinine which previously has been effective in the treatment of severe and complicated disease. In some areas, such as on the Thai/Cambodian and Thai/Myanmar borders, high levels of resistance to mefloquine, the only alternative drug available in control programmes, has developed. Also in parts of Africa, resistance to chloroquine and sulfadoxine-pyrimethamine is intensifying. (Phillips-Howard, 2002)

For regions where multidrug- resistance is prevalent WHO now recommends the artemisinin-based combination therapy (ACT) a combination therapy involving two or three drugs.

Artemisinin, has a very rapid onset of action against the malaria parasite. The combination drug is selected among drugs that have a more persistent effect in preventing the recurrence (recrudescence) of malaria. The combination also gives a greater protection against the development of drug-resistant mutants.

#### Control of Use

Clear guidelines on the use of artemisinin and its derivatives should be produced by national authorities and distributed to all prescribers. This includes national drug formulary and pharmacy guidelines; information should be supplied to health workers to ensure correct prescribing in respect of clinical indication and dosage. Countries in which multidrug-resistance does not exist and which have already registered a drug belonging to the artemisinin group need to seriously reconsider their position, or limit the promotion, marketing and use to the maximum extent possible. (Phillips-Howard, 2002).

To avoid consumer misuse, it is important that the general public as potential or actual patients be educated in the correct use, in particular the importance of compliance, of artemisinin and its derivatives. Care should be taken not to promote the inappropriate use of the drugs. National regulatory agencies should be in a position to ban inaccurate or irregular promotion. Strong education, advertising and government controls are required to restrict their distribution. Promotional campaigns need to be monitored to prevent inaccurate and misleading statements during advertising. A series of mechanisms can facilitate control of the use of these drugs. (Phillips-Howard, 2002).

### 3.2.1 Parts used

Artemisinin, used in the semi-synthesis of related compounds in Artemisinin-based Combination Therapies (ACT), are found mainly in the leaves and flowers of *Artemisia annua*, little artemisinin is found in the stems, and none is found in seeds or roots. (Ferreira et al., 1997).

The leaves are harvested when the artemisinin content is highest and dried leaves are used (with 13% or less water content) for artemisinin extraction.

### 3.2.2 Preparation

The currently available preparations of artemisinin and its derivatives are artesunate for intravenous injection, oral tablets, and suppositories, artemether in oil for intramuscular injection and as oral capsules, arteether for intramuscular injection, and dihydroartemisinin in tablets (Looareesuwan and Wilairatana, 1997).

The most widely available oral preparations are artemisinin tablets, artemether capsules, artesunate (artesunic acid) tablets, and dihydroartemisinin tablets. Intramuscular preparations are: artemether in oil, artesunate as anhydrous powder, and recently a preparation of arteether. The intravenous preparation of artesunate is identical to the preparation for intramuscular injection. Suppository preparations are available for artemisinin, artesunate, and dihydroartemisinin. See Table: The most widely available formulations. (WHO, 1998).

WHO recommends four artemisinin-based combination therapy (ACT)s: artemether-lumefantrine (Coartem®; Novartis), artesunate-mefloquine, artesunate-amodiaquine, and artesunate-sulfadoxine/pyrimethamine.

A WHO Policy Implication sheet outlines the dosage and recommended treatment of artemether-lumefantrine, the same ACT combination used in Coartem®. The artemether-lumefantrine-based combination therapy treatment course consists of tablets containing 20 mg of artemether plus 120 mg of lumefantrine (blenflumetol). (WHO, 2005). Stability tests show that the formulation is stable for 2 years at room temperatures of 25°C and below. The manufacturer recommends that it should not be stored above 30°C. (WHO, 2002).

In semi-immune patients, the manufacturer recommends the 4-dose regimen, consisting of 1, 2, 3 or 4 tablets taken at 0 h, 8 h, 24 h and 48 h. The total course for an adult is 16 tablets, which gives a total dose of 320 mg of artemether plus 1920 mg of lumefantrine.

In areas with multidrug-resistant *P. falciparum* and in non-immune patients, an intensive 6-dose course consisting of the doses shown above at 0 h and 8 h, and twice daily doses on the next 2 days is recommended, see Table.

Thus, the course for an adult would be 4 tablets at 0 h and 8 h and 4 tablets twice a day on the second and third days. (WHO, 2005).

An agreement between WHO and Novartis stipulates that Coartem® will be available to WHO at a no-profit price. At present, the cost of a treatment dose based on the 6-dose regimen is as follows: Children 10-14 kg =US\$ 0.90; Children 15-24 kg =US\$ 1.40; Children 25-34 kg =US\$ 1.90; Adult > 35 kg = US\$ 2.40. This price is approximately half of the current price of the drug currently being used in KwaZulu Natal and equivalent to the cheapest price currently available for the ad hoc combination of artesunate/mefloquine used in Cambodia. The normal price for an adult of artesunate/mefloquine is US\$ 4.06-7.04). (WHO, 2002).

There is no evidence of increased toxicity with the 6-dose as compared to the 4-dose regimen and, for simplicity of implementation, it may be advantageous to use the 6-dose regimen in all areas. (WHO, 2005).

#### Treatment of Uncomplicated Malaria

Artemisinin and its derivatives should be administered as oral therapy in combination with another effective blood schizontocide to reduce recrudescences and to slow the development of resistance. At present data (2002) WHO only support the operational use of the combination with mefloquine (15-25 mg base/kg) but a fixed combination of artemether with lumefrantrine is at an advanced state of development and research on other combinations is also being carried out. Administration of mefloquine on the second or third day considerably reduces the risk of vomiting once the clinical condition has been improved. Tolerance to the 25 mg base/kg doses of mefloquine may be further improved by administering 15 mg base/kg on the second or third day with the rest 6-24 hours later. If compliance is a concern, mefloquine can be given on the first day. The dose of mefloquine depends on the local sensitivity of the parasite to mefloquine.

The following regimens are recommended:

Artemisinin: 20 mg/kg as a divided loading dose on the first day, followed by 10 mg/kg once a day for a further 2 days, plus mefloquine (15-25 mg base/kg) as a single or split dose on the second or third day.

Artesunate: 4 mg/kg once a day for 3 days, plus mefloquine (15-25 mg base/kg) as a single or split dose on the second or third day.

Artemether: 4 mg/kg once a day for 3 days, plus mefloquine (15-25 mg base/kg) as a single or split dose on the second or third day.

The combination of dihydroartemisinin with mefloquine and other drugs is still being evaluated in clinical trials. (Phillips-Howard, 2002)

#### Monotherapy

In those situations where the use of artemisinin combinations is impossible, for example because of patient intolerance to mefloquine, monotherapy with artemisinin drugs may be used in regimens of 7 days with every effort being made to ensure compliance.

Administration of shorter regimens to non-immune patients leads to unacceptably high levels of recrudescences.

The following regimens are recommended:

Artemisinin: 20 mg/kg in a divided loading dose on the first day, followed by 10 mg/kg once a day for 6 days.

Artesunate: 4 mg/kg in a divided loading dose on the first day, followed by 2 mg/kg once a day for 6 days.

Artemether: 4 mg/kg in a divided loading dose on the first day, followed by 2 mg/kg once a day for 6 days.

There are limited data on dihydroartemisinin and further research is required to determine optimal dosage regimens. (Phillips-Howard, 2002)

#### Treatment of Severe and Complicated Malaria

The following schedules are recommended for adults and children over 6 months.

##### Intramuscular artemether

3.2 mg/kg as a loading dose on the first day, followed by 1.6 mg/kg daily for a minimum of 3 days until the patient can take oral therapy of an effective antimalarial. The daily dose of artemether can be given as one single injection. In children, the use of a 1 ml tuberculin syringe is advisable since the injection volumes will be small. A formulation (40 mg/1ml) that is more easily used in children is available from one manufacturer.

##### Intravenous artesunate

2.4 mg/kg as a loading dose on the first day, followed by 1.2 mg/kg daily for a minimum of 3 days until the patient can take oral therapy of an effective antimalarial. The anhydrous acid contents are dissolved in 0.6ml 5% (w/v) sodium hydrogen carbonate. The solution should be prepared just before use, because of the instability of the acid, and be diluted with 5.4 ml of 5% (w/v) dextrose solution or dextrose in normal saline. (Phillips-Howard, 2002)

#### 3.2.3 Constituents

Artemisinin is a sesquiterpene lactone peroxide compound. The endoperoxide linkage is unusual for an antimalarial compound. Structure activity studies carried out indicated that the presence of the peroxide bridge correlates with, and is essential for, the antimalarial activity.

Artemether is the methyl ether derivative of artemisinin, the antimalarial principal extracted from *A. annua*.

Lumefantrine is an aryl amino alcohol similar to quinine, mefloquine and halofantrine. Biochemical studies suggest that its antimalarial effect involves lysosomal trapping of the drug in the intra-erythrocytic parasite, followed by binding to toxic haemin that is produced in the course of haemoglobin digestion. This binding prevents the polymerization of haemin to non-toxic malaria pigment. (WHO, 2005).

#### 3.2.4 Standards

Scientists fear that widespread and indiscriminate use of the artemisinin compounds of varying quality, particularly through self-treatment with the oral formulations, and weak regulatory systems in many countries of the world, may result in a rapid decline in their efficacy. Even where regulatory mechanisms are well established, it is possible that the system maybe by-passed by illegal black market activities, including sub-standard and counterfeit drugs. Unregulated use of artemisinin and its derivatives may result from a variety of factors. These include government failure to enforce regulations to control their

importation and distribution, lack of a national drug policy for antimalarial drugs, uncontrolled importation and distribution, ignorance or lack of cooperation of prescribers on appropriate use of the drugs, lack of cooperation of pharmacists, and unwarranted demand on the part of the public. Irresponsible promotion and advertising by the technical and popular media may influence prescribers, pharmacists and patients (many of the latter having fevers other than malaria) in favour of the use of the artemisinin group of drugs in situations where they are not needed. (Phillips-Howard, 2002).

Pharmacopoeial standards for artemether have been established by WHO, 2003 (International Pharmacopeia, Volume 5, 3rd Edition) but not for lumefantrine or the fixed combination.

### 3.2.5 Methodology

Several methods have been reported for the measurement of artemisinin and its main derivatives in plant material and biological fluids. However, most of them are either not sufficiently sensitive and do not offer reliable results, or are difficult to apply in routine analyses. Therefore, new methods for the determination of these compounds, such as supercritical fluid extraction and chromatography, pressurized solvent extraction, microwave-assisted extraction, high-performance liquid chromatography coupled to mass spectrometry or evaporative light scattering detection, were described by Christen and Veuthey, 2001.

## 3.3 Pharmacology

### 3.3.1 Systems

Artemisia is mentioned in the Chinese Handbook of Prescriptions for Emergency Treatments of 340 AD for treatment of fevers. In 1971, extraction of aerial parts of *A. annua* with low-boiling solvents, such as diethylether, produced a compound mixture with antimalarial properties on infected mice and monkeys. The main active principle, artemisinin (formerly referred to as arteannuin and as qinghaosu in Chinese), was isolated and had its structure correctly defined in 1972 in China as a sesquiterpene lactone with an endoperoxide bridge. Artemisinin is now available commercially in China and Vietnam as an antimalarial drug efficacious against drug-resistant strains of *Plasmodium*, the malarial parasite.

Coartem® (artemether – lumefantrine) is currently registered in 77 countries worldwide and more than seven million patients have benefited from this innovative treatment since its first registration in October 1998. It has been extensively studied in multi-center clinical trials involving more than 3000 patients.

Since 2001, 42 malaria-endemic countries have adopted ACTs: 38 as first-line treatment and 14 as second-line treatment. Of these 42 countries, 23 are in Africa, although only 9 countries were actually implementing ACT treatment policies as of 2004. An additional 14 countries are in the process of changing their malaria treatment policy.

### 3.3.2 Ailments

The artemisinins (term used collectively for artemisinin and its derivatives) compounds are potent and rapidly acting anti-malarials against *Plasmodium falciparum* and *P. vivax*, including multi-drug-resistant strains. (Utzinger et al., 2003). There is less information on the effect against the two other human malaria parasites, *P. malariae* and *P. ovale*, but they are likely to be effective against them as well.

Artemisinin derivatives, dihydroartemisinin, arteether, artemether, artelinic acid and artesunate, are about five times as potent; this outweighs the added cost of derivation, and they are therefore cheaper per treatment course.

Artemisinins rapidly kill the asexual blood stages of the parasites, which are responsible for the disease manifestations (blood schizonticidal activity), they have some effect on the gametocytes (the stage, which is infective to mosquitoes ingesting a blood-meal from an infected person), but have no effect on the hypnozoites, which lodge in the liver and can cause relapses in vivax and ovale malaria. (WHO, 2001).

Pharmacokinetically, the artemisinins are characterized by fast absorption, large distribution volumes and short half-lives. They penetrate the blood-brain barrier, and accumulate in erythrocytes. The mechanism of action is different from that of other anti-malarial medicines and probably related to the release of free radicals, when the peroxide bridge bursts, which happens when the compounds encounter high concentrations of heme, a breakdown product of haemoglobin produced by malaria parasites living in red blood cells. The free radicals rapidly kill the malaria parasites, and the selectivity of their toxicity is probably related to the fact that they only reach high concentrations in the human body in parasitized red blood cells. (Meshnick, 2002). The action of artemisinins is more rapid than that of other known anti-malarials. It has been estimated that treatment with artemisinins reduces the parasite biomass by a factor of approximately 10 for each 36-48 hour asexual cycle of the parasite and by a factor of 106- 108 over a 3-day course of treatment.

Because of the very short half-life, it is believed that, similar to quinine, the artemisinins are unlikely to be affected by the emergence of drug resistance. However, resistance can be elicited in animal models. Also, studies in Yunnan, where artemisinins have been used on a large scale, indicate some reduction of the parasite susceptibility (Yang et al., 2003), a trend which is worrying, although it has not yet been translated to lower clinical efficacy, as far as is known.

Coartem (artemether – lumefantrine) is a highly effective and well tolerated anti-malarial that achieves cure rates of up to 95%, even in areas of multi-drug resistance. It is indicated for the treatment of falciparum malaria, the most dangerous form of malaria.

In addition to antimalarial effects, artemisinin was found to have promise in treating the parasitic diseases schistosomiasis (Utzing et al., 2003) and clonorchiasis (common in China and Africa, affecting over 200 million people each year) caused by trematodes (blood flukes). Artemether is now being used for prophylaxis against schistosomiasis, in combination therapy with praziquantel it is used to treat the disease.

### 3.3.3 Clinical trials

Experimental pharmacology: A total of 16 clinical trials with more than 3000 patients, including 600 children under 5 years of age, have been carried out in Europe, South-East Asia and Africa. In areas with low or no malaria transmission, the 28-day cure rates with a 4-dose regimen were 95.1% outside Thailand and 76.5% in Thailand, where most patients came from areas with multidrug-resistant malaria. In Thailand, a 6-dose regimen gave a 28-day cure rate of 97.3%. In a trial in Africa, the 28-day cure rate complemented by PCR studies to distinguish reinfections from recrudescences showed a corrected cure rate of 92.7% (319). A dose-finding trial in Thailand demonstrated the importance of the number of doses rather than

the dose level for the efficacy of this combination drug. These studies also showed that the cure rate was 97% in patients receiving a total dose of  $>$  or  $=$  50 mg/kg, regardless of the level of initial parasitaemia, but that cure rates were significantly lower with parasite densities of  $>$  or  $=$  20 000 per ml when the total lumefantrine dose was  $<$  50 mg/kg. (WHO, 2005).

A Myanmar study of 141 cerebral malaria cases with intravenous artemisinin plus oral mefloquin resulted in decreased mortality and rapid parasite clearance in comparison to intravenous quinine with oral tetracycline. (Win et al., 1992).

A Vietnam study in 79 comatose cerebral malaria cases demonstrated that there was a rapid reduction in the parasitaemia [16 hrs for artesunate versus 34.5 hrs for intravenous quinine]. But it did not show any significant reduction in the duration of coma or mortality. (Hien et al., 1992).

Patients with moderately severe malaria are likely to be marked by inability to take oral drugs or fluids by mouth. If these patients do not have ready access to treatment by reason of the distance at which they live from a health facility, they will be at considerable risk of deteriorating and dying before getting to a clinic where effective treatment is available. This is how most lives are lost from acute *Plasmodium falciparum* infection, particularly amongst infants and children, and especially in the rural areas of Sub-Saharan Africa.

Data from studies in Africa provides plausible evidence to support the antimalarial efficacy and safety of a single 10 mg/kg dose of artesunate rectal capsules given in the initial management of acute malaria in patients who cannot take medication by mouth and for whom parenteral treatment is not available. The single dose is given in emergency situations when the disease is serious and potentially life-threatening, and will act as an injectable to halt and reverse the progress of disease, achieving clinical stability and recovery in a severely ill patient. (Gomes, 2002).

See table: Clinical trials of artemisinin derivatives either alone or in combination with other antimalaria drugs for adult patients in Thailand.

#### Clinical Results of the Use of *Artemisia annua* Tea - March 2000

The tea is made by pouring one litre of boiling water onto 5 g dried leaves of *Artemisia annua*. It is allowed to brew for 10 to 15 minutes, and then poured through a sieve. This tea is then drunk in four portions in the course of the day. The period of treatment is between 5 and 7 days. Three studies were made in the Democratic Republic of Congo:

Nebobongo (North-east Congo): 48 patients who were admitted to hospital with the classical symptoms of malaria, and who were found to have malaria parasites (all *P. falciparum*) in their blood. After 5 days treatment 44 (92%) had blood free of parasites. 37 patients said they were totally without symptoms, while 11 patients complained of some discomfort, 3 of them had headache, 1 fever, 4 pains in the joints and 1 giddiness. The patients showed no significant side-effects. 11 (25%) reported feelings of sickness, though no patient actually vomited.

Bukuvu (North-east Congo): 91 patients were admitted a health centre with symptoms typical of malaria, and all were shown to have plasmodium in the thick blood smears (59 patients; *P. falciparum*, 15; *P. malariae*, 15; both *P. falciparum* and *P. malariae* and 2; both *P. falciparum* and *P. vivax*). Five of the patients were under 18 years old (17 months, 19 months, and 10, 11,



and 17 years). After 5 days treatment no parasites were to be found in the blood of 86 of the 91 patients (95%). Whilst being treated, 21 patients complained of giddiness, 11 of feelings of sickness, 10 of noises in the ears, 8 of eye problems, 2 of itchininess and 2 of stomachache. 31 of the 92 patients said that they had no new complaints. The thick blood smears were negative following treatment for each of the five children.

Kinshasa: 21 patients were treated with artemisia tea in the Red Cross Health Centre. All patients had symptoms typical of malaria, and each produced a positive thick blood smear. All the patients were over 18 years old. After 7 days treatment with artemisia tea, the blood smears of 19 (91%) of the 21 closely monitored patients were negative. The smears of two patients still contained a few trophozoites. According to the statement of the Health Centre, of the 21 patients, 20 showed a good clinical recovery. (Hirt, 2000).

Fourteen healthy male volunteers received one liter of tea prepared from nine grams of *Artemisia annua* leaves. Blood samples were taken and artemisinin was detected by reversed phase high-performance liquid chromatography. The mean  $\pm$  SD maximum plasma concentration of artemisinin was 240  $\pm$  75 ng/mL and the mean  $\pm$  SD area under the plasma concentration-time curve was 336  $\pm$  71 ng/mL x hr. Artemisinin was absorbed faster from herbal tea preparations than from oral solid dosage forms, but bioavailability was similar. One liter of an aqueous preparation of nine grams of *Artemisia annua* contained 94.5 milligrams of artemisinin (approximately 19% of the usually recommended daily dose). Artemisinin plasma concentrations after intake of this herbal tea are sufficient for clinical effects, but insufficient to recommend such preparations as equivalent substitutes for modern artemisinin drugs in malaria therapy. (Rath et al., 2004).

### 3.3.4 Quality control

The quality of the supplies of artemisinin and its derivatives should be assured to reach internationally recognized standards (WHO, 1992). The licensing of quality drugs can be assured by using the WHO Certification Scheme (WHO, 1992).

In May 2002, in collaboration with other United Nations agencies, WHO established an international mechanism to pre-qualify manufacturers of artemisinin compounds and ACTs on the basis of compliance with internationally recommended standards of manufacturing and quality. Products and manufacturers that meet these standards are included in a list considered acceptable for procurement by United Nations agencies. The list is published as a guide to governments, NGOs and other partners, e.g. the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM), procuring ACTs. To date, one ACT – artemether–lumefantrine (Coartem®) – has been pre-qualified.

WHO and UNICEF have called for tenders of co-blistered combinations of the following products for which there are not yet pre-qualified manufacturers: 1) artesunate plus amodiaquine; 2) artesunate plus sulfadoxine/pyrimethamine; 3) artesunate plus mefloquine; and 4) amodiaquine plus sulfadoxine/pyrimethamine. (WHO, 2005).

### 3.3.5 Precautions

Coartem® (artemether – lumefantrine) is not recommended for chemoprophylaxis.

Parasite resistance

There is no convincing evidence yet of clinically relevant, stable, parasite resistance having developed to artesunate, or to the other artemisinins. Resistant strains of fresh *P. falciparum* have been established although not sustained in vitro. (Inselburg, 1985).

There is however serious concern that the indiscriminate use and misuse of these drugs may encourage the development of parasite resistance. Monitoring of resistance is a responsibility of national malaria control programmes and should be conducted at sentinel sites on a regular repetitive schedule. It is advisable that the clinical progress of patients treated in hospitals be carefully documented in order to detect, as early as possible, any drug failure that might be an early sign of resistance. (Phillips-Howard, 2002).

#### Use in pregnancy

Artemether-lumefantrine is contraindicated in: pregnant and lactating women (Safety of its use in pregnancy has not yet been established.), persons with known hypersensitivity to either of the components, persons with severe malaria. (WHO, 2005).

Artemisinins are not recommended for use in the first trimester of pregnancy, unless they are of life-saving importance for the mother. According to WHO, they can be given in the second and third trimester, if no suitable alternate is available. (WHO, 2003).

In view of the serious health implications of malaria in pregnancy, artemisinin and related compounds should not be withheld from pregnant women in areas where these drugs are indicated. For the management of uncomplicated malaria in pregnancy, artemisinin and its derivatives can be used in the second and third trimester, but their use in the first trimester is not recommended. For the treatment of severe malaria in the first trimester, the advantages of artemisinin drugs over quinine, especially the lower risk of hypoglycaemia, must be weighed against the fact that there is still limited documentation on pregnancy outcomes following their use. The inadequacy of current knowledge on the use of these drugs during pregnancy should be understood by care providers, and if possible, all pregnancies exposed to these drugs should be monitored. Reports of all clinical outcomes, both successful and adverse events should be made to regulatory authorities. (Phillips-Howard, 2002).

#### Side effects

Side effects of artemisinin treatments at normal to high therapeutic doses appear to be rare and mostly involve gastro-intestinal reactions such as dizziness and fatigue, anorexia, nausea, vomiting, abdominal pain, palpitations, myalgia, sleep disorders, arthralgia, headache, rash, and diarrhea (with or without intestinal cramping). In a large study in Thailand comparing high-dose artemisinin derivatives (artemether and artesunate) alone versus in combination with mefloquine, the incidence of adverse effects with the artemisinin compounds was reported to be 34% for loss of appetite, 16% for nausea, 15% for dizziness, and 11% for vomiting. Mefloquine greatly increased the incidence of side effects, doubling the rate. In a clinical trial comparing artesunate injection with chloroquine and with the combination of quinine and resorcin, no adverse effects of artesunate were reported, while dizziness was a common complaint with the drug therapies.

#### Counterfeit products

Fake artesunate is being distributed worldwide with potential lethal results if used to treat *Plasmodium falciparum* malaria. A field method has been developed to test the artesunate content of tablets. (Green et al., 1999). Regional collaboration is essential in respect of product marketing and registration, because tight regulations in one country and not in its

neighbours will result in the development of black market smuggling which inevitably leads to sales of substandard or counterfeit products. (Phillips-Howard, 2002).

Drug regulatory authorities should be informed that most artemisinin group drugs are not produced to GMP standards, and that in some countries drugs of substandard quality are being promoted and used. Governments should promote measures to reduce black market sales and counterfeit drugs and take care in the selection of products for registration, assessing the need and assuring quality, efficacy and safety. It is important to elicit the support of pharmacists and practitioners in peripheral health areas to ensure proper control of distribution and prevent use of counterfeit drugs. (Phillips-Howard, 2002).

### 3.3.6 Toxicology

Toxicities are unknown but the pollen may be extremely allergenic as it is in other species of *Artemisia*. (Duke, 1983). Skin contact with the plant can cause dermatitis or other allergic reactions in some people. Likely to be toxic as are other members of the genus. (Janick, 1995). The taste is bitterly aromatic, strongest when grown in dry, poor soil in full sun.

Well-documented clinical uses of Artemisinin and derivatives have shown few insignificant side effects. However, intra-muscular rats' brainstem studies have shown that intramuscular arteether at 25-mg/Kg/day resulted in rats' brainstem pathology with damage to the auditory nuclei. Parenteral artemether in the treatment of cerebral malaria in Gambien children shows a prolonged recovery from coma and post-treatment convulsions than the same treatment with quinine. Also in Vietnamese adults, a prolonged recovery was noted, but no increased in mortality or neurological behaviour afterwards (Heppner and Ballou, 1998). Others are transient heart block, transient decrease in blood neutrophils and brief episodes of fever.

In experimental animals severe neurotoxic effects have been induced by the administration of very high, prolonged doses, but there is no definitive evidence of neurotoxicity in humans. In rodents and other experimental animals, foetal resorption has been observed, when these drugs have been given in relatively low doses after the sixth day of gestation, and in some species malformations have been observed after exposure in early pregnancy. (WHO, 2003).

## 3.4 Wild harvesting

### 3.4.1 Methodology

In China and Vietnam whole plants are cut from wild populations just before flowering when the leaf artemisinin content is higher. This implies a threat to the genetic resource base as seed, of course, will not be produced.

### 3.4.2 Legislation

As far as is known, there are no regulations on wild harvesting *A. annua* in China now. In Guangxi, farmers go to the mountain to harvest wild *A. annua* and sell it to traders and traders sell it to the pharmacy. This is the problem, if this situation continues, it will result in decrease of wild *A. annua* seed year after year and exhaustion of the wild resources. So the resources protection and artificial cultivation are very important for *A. annua* sustained production. (Pers. Comm., September 2005 from Dr. Dr. Qizhan Tang GXAAS, China).

### 3.4.3 Conservation

### 3.5 Marketing

Artemisinin formulations are manufactured in Vietnam (several establishments in Hanoi and Ho Chi Minh City), artesunate in Vietnam (Central Pharmaceutical Enterprise II in Hanoi) and in China (Guilin Pharmaceutical Works), and artemether in China (Kunming Pharmaceutical Factory). A Belgian company, Profarma, imports raw materials from Vietnam and then manufactures oral and intramuscular artemether. (Phillips-Howard, 2002).

Registration and marketing authorization of artemisinin and its derivatives should be restricted to areas where multi drug-resistance is prevalent, i.e., where chloroquine and, sulfapyrimethamine combinations and quinine are ineffective. At present, such areas are found in Brazil, Cambodia, China, Lao Peoples' Democratic Republic, Myanmar, Thailand and Vietnam. (Phillips-Howard, 2002). Each artemisinin product should be registered nationally as a prerequisite for routine use (Phillips-Howard, 1994). Drug licencing should only occur when it is deemed essential for use, with a description for its correct use in the national drug policy. All drugs should be registered according to strict guidelines and require a product licence (WHO, 1990). For imported products the licensing process can be simplified by efficient use of the WHO Certification Scheme of the Quality of Pharmaceutical Products Moving in International Commerce (WHO, 1992). If a product has been registered and produced in a country having a reputable drug regulatory authority, a WHO-type certificate might provide sufficient basis for issuing a marketing licence. WHO has offered to advise national authorities regarding local manufacturing facilities (WHO, 1994).

Importation of artemisinin and its derivatives should be controlled by national governments through a governmental health authority, a central drug procurement agency or by a limited number of licensed importers. In the absence of a national regulatory system or authority for drug registration, minimum requirements should include the use of the WHO Certification Scheme to obtain information on the regulatory status of the exporting country.

It is recommended that regulatory authorities in exporting countries provide information to regulatory authorities in importing countries in respect of the quality and regulatory status of the drugs. Where the drugs are used, routine sampling of artemisinin and its derivatives should be carried out to monitor their quality and the quality assurance procedure should be verified by independent experts. Screening should be routinely performed at port of entry (if imported) or at the factory, and again at the point of distribution to identify drug degradation following transit and storage.

Withdrawal of the whole drug batch is mandatory for drugs not reaching predefined standards, and if the quality of drugs is consistently sub-optimal. Where laboratory facilities are able to monitor drug quality, information on quality control specifications from the pharmaceutical company manufacturing the drugs is required. The specifications file includes the drugs chemical formulation, the manufacturing process, assay techniques, and quality assurance procedures. There are currently three methods of assessment of drug quality. Quality assurance may best be established as regional or interregional reference laboratories to serve all countries in the region. (Phillips-Howard, 2002)

#### Post-Marketing Surveillance (PMS)

The principal objectives of post-marketing surveillance are to identify problems related to drug quality, stability, and efficacy, adverse reactions that may be of a delayed nature, and the development of drug resistance. Such problems may not have been recognized in the initial

trials of the drugs, or were not communicated to the manufacturer. Ideally, these should be carefully monitored by each national malaria control programme on a general basis and possibly through more detailed monitoring at sentinel sites. No standardized methods exist yet for monitoring therapeutic efficacy or resistance to this group of drugs in the field and these urgently need to be established. (Phillips-Howard, 2002).

### 3.6 Cultivation

### 3.7 Notes

#### Further research

See table on recommended further research and studies of artemisinin, its derivatives and anti-malaria properties. (Phillips-Howard, 2002).

#### Contacts:

This record is under construction (November 2005) in collaboration with WHO:.....

As well as various Internet sites consulted (see the "Links" icon) information has been supplied personally by Allan Schapira, WHO; James Simon, Rutgers/ASNAPP; James Duke, ARS; Barney Gasston, Africa Bio-Medica Ltd. & EABL; Qizhan Tang, Gaungxi Academy of Agricultural Sciences (GXAAS, China); Nguyen Van Thuan, Vietnam; Anil Singh, CIMAP, India; José Abramo Marchese, Federal University for Technology of Paraná, Brazil; Pedro M. de Magalhães, UNICAMP/CPQBA, Brazil; John Laughlin Tasmania, Australia; Dan Gudahl, Winrock International (re. Cameroon); Daw Khin Phyu Phyu, Myanmar; to be continued with more contacts and contact data.....

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WHO (2003) Guidelines for Good Agricultural and Collection Practices (GACP) for Medicinal Plants. (See cover).

Wright C.W. (Ed.). (2002) *Artemisia*. (See cover).

### 3.9 Links

Purdue University's NewCROP programme

[Purdue University's NewCROP programme](#)

[Purdue University's NewCROP programme](#)

[Plants For A Future](#)

[Agricultural Research Service - Dr. Duke's Phytochemical and Ethnobotanical Databases](#)

[WHO facts on Artemisinin-based Combination Therapies](#)

[WHO Roll Back Malaria Department](#)

[WHO guidelines on good agricultural and collection practices \(GACP\) for medicinal plants](#)

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