WHO monograph

on good agricultural and collection practices (GACP) for Artemisia annua L.
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(GACP)
for *Artemisia annua* L.
Acknowledgements

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Preface

Traditional medicine, a valuable treasure of the Chinese nation, is an important component of the medical science in China. In the thousands of years of Chinese history, traditional medicine has played a significant role and made an indelible contribution to the health of the Chinese people. During the last two decades, traditional Chinese medicine has been increasingly accepted by many other countries. A good example is acupuncture, which is being used for clinical treatment in most of the countries in the world.

Since the founding of the People’s Republic of China, the Government of China has made unswerving efforts to promote the development of traditional Chinese medicine, which has become an important component of the contemporary medical medicine. The extraction of artemisinin from traditional herbs as a very efficacious antimalaria drug, is an example of the successful combination of traditional Chinese medicine and modern medicine.

Today in the 21st century, malaria, the ancient disease, is still severely challenging people’s health. Each year, more than one million people around the globe die of malaria and more than two billion people in over 100 countries and regions are threatened by the disease. In many developing countries, especially those in Africa, the morbidity and mortality from malaria are still very high. Malaria has had serious impact on the social and economic development of mankind. In order to effectively curb the spread of malaria in the world, WHO has recommended the use of artemisinin-based combination therapies (ACT) for the treatment of this disease.

In September 2005, the Chinese President Mr Hu Jintao pronounced at the 2005 World Summit that China will strengthen its cooperation with developing nations in terms of prevention and control of HIV/AIDS, malaria and other infectious diseases, and increase relevant assistance to developing countries, especially African countries.

China is the place of origin of Artemisia annua L., the largest supplier country of Artemisia annua L raw material on the global market, and the first country to extract artemisinin. And artemisinin is a significant contribution of traditional Chinese medicine to the world. China not only has a wealth of experience in the production and usage of artemisinin, but also has unique skills in the cultivation of Artemisia annua L. We stand ready to strengthen exchange and cooperation with WHO and relevant countries in treating malaria using artemisinin, and to make a vigorous contribution to curbing the spread of malaria in the world.
China actively supported WHO in developing this monograph on the cultivation of *Artemisia annua* L. It is hoped that the publication of this monograph will be helpful for the prevention and treatment of malaria around the globe. We are willing to continue working together with other countries and WHO in promoting traditional Chinese medicine to serve the health of the people around the world.

Mr Gao Qiang  
Minister of Health  
People's Republic of China
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1. Introduction

1.1 Background

_Artemisia annua_ L. is a traditional Chinese medicine. It has been used in China for more than 2000 years for treating many disorders including malaria. The isolation of artemisinin from _Artemisia annua_ L. and the characterization of its antimalarial effect in the 1970s was initiated by Chinese scientists. This was one of the most important advances in malaria control in modern times.

The WHO Roll Back Malaria department has reported that recent estimates of the global malaria burden have shown increasing levels of malaria morbidity and mortality, reflecting the deterioration of the malaria situation in Africa during the 1990s. About 90% of all deaths from malaria occur in Africa, in the areas south of the Sahara, and the great majority of these are in children under the age of five years.

Key among the factors contributing to the increasing malaria mortality and morbidity is the widespread resistance of _Plasmodium falciparum_ to conventional antimalarial drugs, such as chloroquine, sulfadoxine–pyrimethamine (SP) and amodiaquine. Multidrug-resistant _P. falciparum_ malaria is widely prevalent in south-east Asia and South America.

In response to increasing levels of antimalarial resistance, since 2001 WHO has recommended that all countries experiencing resistance should use combination therapies instead of conventional monotherapies; preferably antimalaria medicines in combination with artemisinin derivatives (artemisinin-based combination therapies (ACTs)) for falciparum malaria. Therefore the world market for products including artemisinin derivatives is now growing rapidly, and the demand for artemisinin is increasing.

At present, artemisinin compounds are derived from a raw substance extracted from the plant _Artemisia annua_ L. Cultivation of this plant requires a minimum of 6 months, and extraction, processing and manufacturing of the final product require at least 2–5 months depending on the product formulation. Agricultural production is not a problem or limiting factor. However, if the rapid increase in demand for the pharmaceutical products is not predicted in time to allow for increased agricultural production, there could be temporary shortages in supply. Reliable forecasting of global ACT requirements is thus essential.

With this in mind, the Traditional Medicine team and the Roll Back Malaria department have worked together to develop this model monograph on good agricultural practices for _Artemisia annua_ L. to facilitate cultivation of this particular medicinal plant with reliable quality and to ensure a sustainable supply to meet market demands.
1.2 Objectives

The objectives of this model monograph are to:
♦ provide practical and specific technical guidance on the cultivation and collection of *Artemisia annua* L. of good quality and a high yield;
♦ contribute to alleviating the shortage of artemisinin-based medicines;
♦ provide a model monograph as a basis for countries and researchers to develop further monographs on good agricultural and collection practices (GACP) for other medicinal plants;
♦ protect the wild resources of medicinal plants, in particular *Artemisia annua* L., to ensure their sustainable use.

1.2.1 Utilization

The monograph provides a detailed description of the techniques and measures required for the cultivation and collection of *Artemisia annua* L. This information was gathered not only from research data but also from the practical experience of several countries where successful cultivation practices have led to a high yield of good quality *Artemisia annua* L. The cultivation of high-yield, good quality medicinal plants depends on a number of factors such as the geographical environment, altitude, temperature, rainfall and soil characteristics. Therefore this monograph should be used in conjunction with other WHO documents and publications relating to the quality assurance of herbal medicines and the conservation of medicinal plants, for example, *WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants* (1).

During the preparation of this monograph, we also found that experiences in the cultivation of *Artemisia annua* L. vary considerably from country to country and the growth of the plants depends not only on the techniques and measures used, but also on the country’s natural and geographical conditions. These differences are also covered in the monograph, but it should be noted that the techniques and measures introduced in this document are for reference only.

Besides the technical issues, the following key issues also need to be carefully considered.

1.2.2 Pilot test of cultivation in a small area

*Artemisia annua* L. is grown worldwide; however, this does not mean that all the plants necessarily contain artemisinin, which is the effective ingredient for anti-malaria treatment. In some places, although *Artemisia annua* L. may contain artemisinin, the content may be very low and without industrial value. These factors, together with the above-mentioned complicated technical and environmental factors (e.g. altitude, temperature, rainfall, soil characteristics and method of cultivation), mean that a pilot test of cultivation on a small area of land is necessary to ensure that the cultivation areas selected are suitable for growing high-yield plants before large-scale cultivation begins.

1.2.3 Estimation of market demand and potential clients

The effective ingredient of *Artemisia annua* L. – artemisin – is not very stable and this is very clearly stated in this monograph. This is because the special
peroxide group in its chemical structure makes artemisinin unstable when heated. It decomposes easily if stored in warm, humid conditions where deoxidizing substances are present. High temperatures should therefore be avoided during postharvest handling. After harvesting or collection, the artemisinin content of the leaves will gradually decrease. The value as a raw material for extraction, would be lost after 1 year's storage. According to the experience of some countries, the raw materials may lose their industrial value 6 months after harvest or collection. Therefore, before extensive cultivation begins, it is important to estimate the market demand and to identify potential clients for the plant materials.

1.2.4 Ensuring the extracting capacity for artemisinin from locally grown plants

In some countries, where high-yield plants of *Artemisia annua* L. grow, the governments may want to produce artemisinin-based medicines themselves. WHO encourages and supports developing countries in promoting their local medicinal products, but would also like to draw the attention of governments to the need to establish whether the technical skills and know-how needed to extract artemisinin from dried leaves are locally available before large-scale cultivation begins.

Dr Xiaorui Zhang
Coordinator
Traditional Medicine
Department of Technical Cooperation
for Essential Drugs and Traditional Medicines
World Health Organization
2. Botanical and pharmacological characteristics of *Artemisia annua* L.

2.1 Name of plant

2.1.1 Scientific name

Latin binomial name: *Artemisia annua* L.
Family: Asteraceae (Compositae)

*Artemisia annua* L. is abbreviated to *A. annua* throughout this document.

2.1.2 Selected vernacular names

Chinese: Caohao, Cao Qinghao, Cao Haozi, Chouhao, Chou Qinghao, Haozi, Jiu Bingcao, Kuhao, San Gengcao, Xianghao, Xiang Qinghao, Xiang Sicao, Xiyehao (2, 3).

English: annual wormwood, sweet wormwood (4).

French: armoise annuelle (5).

Japanese: Kusoninjin (6).

Korean: Chui-ho, Hwang-hwa-ho, Gae-tong-sook (6-8).

Vietnamese: Thanh cao hoa vàng (5).

2.2 Medicinal plant part of interest

2.2.1 Part used in traditional medicine

Dried aerial parts.

The aerial parts have been used in traditional Chinese medicine for the treatment of: malaria; fever caused by tuberculosis; jaundice; "fever caused by summer-heat"; and "afternoon fever related to deficiency of yin" (2).

2.2.2 Part to be used as raw material for the extraction of artemisinin

Dried leaves.

---

1 Asteraceae are also known as Compositae.
2.3 Geographical distribution and the major areas of cultivation

2.3.1 Geographical distribution

*A. annua* is widely distributed in the temperate, cool temperate and subtropical zones (mainly in Asia) of the world. It originated from China and grows mainly in the middle, eastern and southern parts of Europe and in the northern, middle and eastern parts of Asia. However, it also grows in the Mediterranean region and countries in North Africa, as well as in south and south-west Asia. In addition, following its settlement in North America from northern Asia, it grows widely in Canada and the United States (9).

2.3.2 Major areas of cultivation

A few countries are currently cultivating *A. annua* on a large scale, such as China, Kenya, the United Republic of Tanzania and Viet Nam. Small-scale cultivation has been undertaken in India and other countries in Africa, south Europe and south America. *A. annua* for industrial use is mainly collected from the wild.

2.4 Morphological characteristics of live *A. annua*

Annual plant, aromatic, green, glabrous or with scattered, small, approximate hairs. Stem erect, ribbed, brownish or violet-brown, naturally grows to 30–100 cm high (cultivated plants may reach 200 cm high). Leaves alveolate-punctate-glandular; lower leaves petiolate, 3–5 cm long and 2–4 cm wide, ovate, thrice pinnately cut, their lobules oblong-lanceolate, short-acuminate, entire or with 1–2 teeth, 1–2 mm long and 0.5 mm wide; middle and cauline leaves twice pinnately cut; upper leaves sessile, smaller and less compound; uppermost leaves bracteal, simple with fewer lateral lobes. Capitula globose, 2.0–2.5 mm in diameter, numerous, divergent or drooping, on short peduncles, approximate on short branches, usually in long pyramidal paniculate inflorescence. Involucre glabrous. Outer involucral bracts linear-oblong, green; inner oval or almost round, with wide scarious border, lustrous. Receptacle convex, glabrous. Peripheral florets pistillate, 10–20, filiform, punctate-glandular; their stigma lobes narrowly linear, obtuse, exserted from corolla tube; disk florets bisexual, 10–30, their corollas cup-shaped-tubular, glabrous; anthers narrowly linear, apical appendages of anthers long, acute, basal appendages very short, subacute; style shorter than stamens, stigma lobes linear, straight, weakly divergent, apically ciliate. Achenes 0.6–0.8 mm long, oblong-ovate, flattened, with small round areola at apex, scariously bordered (10).

2.5 General description of characteristics of plant material

There is a long history of use of the aerial parts and leaves of *A. annua* for medicinal purposes in certain Asian countries. The aerial part of *A. annua* is used as a traditional medicine, whereas the leaves are utilized as raw materials for the extraction of artemisinin.
**Dried aerial parts**

Stems cylindrical, frequently branched at the upper part, 30–80 cm long, 2–6 mm in diameter; externally yellowish green or brownish yellow, with longitudinal ridges; texture slightly hard, easily broken, fracture medullated in the centre. Leaves alternate, dark green or brownish-green, rolled and crumpled, easily broken, when whole, tri-pinnatipartite, the segments and smaller segments oblong or long-elliptical, pubescent on both surfaces. Odour, characteristically aromatic; taste, slightly bitter (2).

**Dried leaves**

Plant material composed of *A. annua* leaves is fragile and green or brown-green in powder form, with a characteristic fragrance. It tastes slightly bitter and refreshing. When examined under the microscope, the cells of the upper and lower epidermis are irregular in shape: the epidermal cells on vein ribs are narrow oblong, and anomocytic type stomata protrude slightly from the surface. The surface is covered with dense glandular and non-glandular hairs, non-glandular hairs mostly converge in the vicinity of the midrib, with much T-shaped hair, and its parietal cells extend horizontally or fold to a V-shape at the point of insertion on the leaf-stalk; the leaf-stalk consists of 3–8 cells and is uniseriate, the basal stalk cells are about 2–3 times bigger than other cells. Parietal cells fall off easily, glandular hairs oval and usually full of light yellow essential oil, and two semicircular secretory cells arrayed in opposition (11).

### 2.6 Chemical composition

#### 2.6.1 The major chemical constituents of *A. annua*

The chemical composition of *A. annua* consists of volatile and non-volatile constituents. The volatile components are mainly attributable to essential oils with the content of the latter being 0.2–0.25%. The main compounds, which account for about 70% of the essential oils, appear to be camphene, β-camphene, isoartemisia ketone, 1-camphor, β-caryophyllene and β-pinene. In addition, other minor ingredients, such as artemisia ketone, 1,8-cineole, camphene hydrate, and cuminal are also found in the volatile parts of *A. annua*. The main non-volatile ingredients include sesquiterpenoids, flavonoids and coumarins, together with proteins (such as β-galactosidase, β-glucosidase), steroids (e.g. β-sitosterol and stigmasterol) (11–13).

The main chemical constituents of *A. annua* are sesquiterpenoids, including artemisinin, artemisinin I, artemisinin II, artemisinin III, artemisinin IV, artemisinin V, artemisic acid, artemisilactone, artemisinol and epoxyarteannuinic acid (11, 14, 15).
2.6.2 Physical and chemical properties of artemisinin (16)

Chemical structure:

Molecular formula: C_{15}H_{22}O_{5}

Chemical name:
(3R,5αS,6R,8αS,9R,12S,12αR)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10(3H)-one

Relative molecular mass: 282.3

Description: colourless needles or a white, crystalline powder.

Solubility: Practically insoluble in water.

Storage: Artemisinin should be kept in a well-closed container, protected from light and stored in a cool place.

Melting range: 151–154 °C.

Specific optical rotation: Use a 10 mg/ml solution in dehydrated ethanol;
\[ [\alpha]_D^{20^\circ} = +75^\circ \text{ to } +78^\circ \]

For a detailed quality specification of artemisinin, see Annex 2 and refer to the International pharmacopoeia (16).

2.6.3 Quality specification of artemisinin derivatives

Artemether and artesunate are the major chemical derivatives of artemisinin. Quality specifications for the above-mentioned substances are described in monographs included in the International pharmacopoeia (3rd ed., vol. 5) (16). For further details, see Annex 2 and refer to the International pharmacopoeia (16).

2.7 Pharmacological activity

*A. annua* has a long history of medicinal use in China and also has various uses in other countries.
2.7.1 Antimalarial activity

*A. annua*

The *Pharmacopoeia of the People’s Republic of China* has registered its indication: malaria with chills and fever; and its action: to stop malarial attacks (2).

*Artemisinin*

Artemisinin, a sesquiterpene lactone endoperoxide extracted and isolated from the leaves of *A. annua*, is an antimalarial medicine (17, 18).

*Artemisinin derivatives*

Artelinic acid, artemether, artemotil (arteether, β-arteether) artemimol (dihydroartemisinin, β-dihydroartemisinin) and artesunate, are considered to be about five times more potent than artemisinin (18).

*Artemisinin compounds* (collective term used for artemisinin and its derivatives) (18)

The artemisinin compounds are effective against *Plasmodium falciparum* and *P. vivax*, including multidrug-resistant strains. There is less information on the effectiveness against the two other human malaria parasites, *P. malariae* and *P. ovale*, but they are likely to be effective against these parasites as well. They 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.

2.7.2 Other activities and uses

*A. annua*

The *Pharmacopoeia of the People’s Republic of China* lists the following as indications: fever caused by summer-heat; afternoon fever related to deficiency of yin or in consumptive diseases; and jaundice. It also describes its action as “to remove summer-heat”, and to relieve consumptive fever (2).

*A. annua* has analgesic–antipyretic effects (19). *A. annua* has also been found to have antibacterial (3) and anti-inflammatory activities (20).

The essential oil of *A. annua* has been reported to have repellent effects on certain beetles (21).

*Artemisinin*

Artemisinin has been reported to be a potent plant growth inhibitor with potential as a natural herbicide (22). Effects on the immune system have also been reported (19), including immunosuppressive activity both in vivo and in vitro (23). Inhibitory activity on transformed oral epithelial cells has been demonstrated in vitro (24).

*Artemisinin derivatives*

Artemisinin derivatives have also been found to be effective against schistosomiasis (25), and there is some evidence that they may have an effect against arrhythmia in rats (26). In vitro studies have indicated
immunosuppressive activity of a novel dihydroartemisinin derivative (27). In vitro studies have also suggested inhibitory effects on various cancer cells (28–30), and on hepatitis B virus (HBV) in vitro (31).

2.8 Clinical applications

*A. annua*

To remove summer-heat, relieve consumptive fever, and treat malarial attacks.

Usage and dosage for the above purposes:

6–12 g, added to the decoction when it is nearly done (2).

It has been reported in China that *A. annua* has analgesic–antipyretic effects and is effective against lupus erythematosus and oral mucosa lichen planus (19).

Dosage forms and doses for the above purposes are given below:

- **Analgesic–antipyretic**: 25–30 g dried herb boiled for less than 30 minutes to make a decoction: take once per day, for 7 days.
- **Lupus erythematosus**: use honey and the fine-ground powder of *A. annua* to make a pill: take 36–54 g per day, for 2–3 months.
- **Oral mucosa lichen planus**: use honey and the ground powdered herb to make a pill (9 g/pill): take 4–6 pills per day, for 1–3 months.

**Artemisinin compounds**

*Malaria*

Artemisinin compounds have proven to be potent and effective medicines for the treatment of malaria, including cerebral malaria and multidrug-resistant falciparum malaria. Currently, the following compounds and the respective dosage forms, indicated with asterisks, are included as antimalarial medicines, in the WHO Model List of Essential Medicines (14th ed, revised March 2005) (32) and monographs on the medicines listed below are included in the *International Pharmacopoeia*, 3rd ed. (16).

- ♦ artemether; artemether capsules; artemether tablets;* artemether injection*
- ♦ artemisinin; artemisinin capsules; artemisinin tablets
- ♦ artemetil (artether, β-arteether); artemetil injection
- ♦ artenimol (dihydroartemisinin, β-dihydroartemisinin); artenimol tablets
- ♦ artesunate; artesunate tablets

For details of the dosage forms and doses, as well as their quality specification see references 16 and 32.

*Schistosomiasis*

The artemisinin compounds are mainly effective against the juvenile schistosome stages (25) in China. Controlled clinical trials have found artemether and artesunate to be effective for the prevention of *Schistosoma japonicum* infection (33), but these findings have not yet translated to large-scale public health use.
3. Good agricultural practices

3.1 Growth and development characteristics

3.1.1 General description

The growth cycle of *A. annua* from sowing to withering consists of six stages, namely the **seedling**, **branching**, **flower-budding**, **flowering**, **fruiting** and **withering** phases. The length of each growth period of *A. annua* varies with seed source, cultivation techniques, site and conditions. Following emergence, the biomass of *A. annua* increases with the increase in the length of growth period and reaches its maximum before flower budding. Biomass decreases from the time of flowering to plant withering. Examples of the duration of each growth phase in different cultivation sites are shown in Table 1.

*Table 1: Growth of *A. annua* in selected cultivation sites*

<table>
<thead>
<tr>
<th>Growth phase</th>
<th>China</th>
<th>Vietnam</th>
<th>Kenya and United Republic of Tanzania</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed germination</td>
<td>7-10 days after sowing</td>
<td>8-16 days after sowing</td>
<td>4-10 days after sowing</td>
</tr>
<tr>
<td>Appearance of 1st pair of leaves</td>
<td>Further 7-15 days</td>
<td>Further 8-15 days</td>
<td>Further 7-15 days</td>
</tr>
<tr>
<td>Appearance of 2nd pair of leaves</td>
<td>Next 15-25 days</td>
<td>Next 16-22 days</td>
<td>Next 15-25 days</td>
</tr>
<tr>
<td>Branching</td>
<td>60-75 days after transplantation</td>
<td>50-69 days after transplantation</td>
<td>60-100 days after transplantation</td>
</tr>
<tr>
<td>Cessation of growth in height</td>
<td>170 days after transplantation</td>
<td>165 days after transplantation</td>
<td>180 days after transplantation</td>
</tr>
<tr>
<td>Flowering</td>
<td>190 days after transplantation</td>
<td>195 days after transplantation</td>
<td>240 days after transplantation</td>
</tr>
<tr>
<td>Full fruition</td>
<td>235 days after transplantation</td>
<td>230 days after transplantation</td>
<td>240 days after transplantation</td>
</tr>
<tr>
<td>Withering</td>
<td>260 days after transplantation</td>
<td>255 days after transplantation</td>
<td>250-260 days after transplantation</td>
</tr>
</tbody>
</table>
3.1.2 Photoperiod

*Artemisia annua* is a short-day plant. In its branching phase the plant is very sensitive to short photoperiodic stimulus and flowers about 2 weeks after induction. Studies demonstrate that the photoperiod of *A. annua* is about 13.5 hours (34, 35).

Because the daylength varies between countries and regions, it is crucial to plant *A. annua* at the appropriate time, otherwise, the biomass and content of artemisinin will be reduced. Each growth and development stage is affected by the number of hours of sunlight. *A. annua* will flower too early if the period of illumination is prolonged. In contrast, a reduction in the number of hours of illumination will lead to delayed flowering. Preliminary trials related to the conditions for the cultivation of *A. annua* are therefore warranted before any large-scale production begins.

3.2 Preferred growing conditions

3.2.1 Ecological conditions

*A. annua* has high adaptability with a proclivity for sunny conditions. The temperature for seed germination is above 7 °C. Based on the experiences in various countries, the optimal growth temperature has been found to be 20–25 °C. *A. annua* is distributed on hillsides and grows well on the edges of forests and on wasteland. The altitude at which it grows varies from country to country, for example, 50–500 m in Viet Nam, 600–800 m in China and 1000–1500 m in Kenya and the United Republic of Tanzania.

3.2.2 Climatic conditions

*A. annua* grows well in warm climates. Experience has shown that it requires an annual average air temperature in the range from 13.5 to 17.5 °C; the accumulated temperature which is not lower than 10 °C should be between 3500 and 5000 °C (to be determined for individual situations), and the annual number of hours of sunlight should be about 1000 hours. The most suitable conditions for the growth of *A. annua* are found in tropical humid monsoon climatic regions, where the average temperature during the growth phase is 17.6–28.4 °C and the annual rainfall is 1150–1350 mm, of which 600–1000 mm is needed during the growing season.

3.2.3 Soil conditions

*A. annua* grows in most soil types provided that the pH of the soil is between 4.5 and 8.5 and it has a deep topsoil and good drainage properties (35).

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2 Accumulated temperature is the sum of mean daily air temperatures in °C over a specified period of time. It is an important index indicating both the temperature conditions required for the development and growth of the plant, and for evaluating heating resources.
3.2.4 Nutrient conditions

For *A. annua*, the maximum absorption of nitrogen occurs during the initial branching and alabastrum stages, and that of phosphate occurs prior to the initial branching and blooming stages, whereas the absorption of potassium increases linearly from the seedling to alabastrum stages. Therefore, potassium should be used as the base fertilizer.

3.2.5 Water conditions

Prior to the appearance of the sixth leaf blades, *A. annua* seedlings are susceptible to drought or waterlogging. Once established, the plants exhibit strong adaptability and resistance to drought or waterlogging, because of their abundant and dense lateral roots. However, *A. annua* has relatively strict requirements for water supply when the seedlings are young and during the preliminary phase of growth. As a result, sufficient water supply and/or the necessary drainage should be ensured in this period (36).

3.3 Seeds

3.3.1 Seeds and cultivar

Seeds should originate from plants of the species of *A. annua* (Asteraceae). Seeds of other origins show a marked difference in the germplasm with artemisinin content ranging from 0.01% to more than 1.0% (37). Therefore, it is necessary to know the origin of the seed and the propagation materials prior to the cultivation of *A. annua*, so as to confirm that the propagation materials or seeds are suitable for the selected site of cultivation. The optimum cultivation schedule should be based on the characteristics of the propagation materials or seeds chosen.

3.3.2 Morphology of the seeds of *A. annua*

The fruit of *A. annua* is an achene with a single seed inside. The seeds are approximately 1 mm in length, oblong, yellow-brownish with a lustrous surface marked by vertical furrows; seed endosperm is creamy white in colour and fatty in content.

The seeds weigh approximately 0.03 g per 1000 (36). The best *A. annua* seeds are full and even in shape. The seeds can be stored for an average period of 4 months if the water content is less than 13%.

3.3.3 Propagation

*A. annua* propagates by seeds. Cultivars with optimal growth characteristics are selected for collection of seeds according to the local growing season. In general, 60–120 g of seeds can be collected from each cultivar. The seeds do not have a dormant phase and can be used in the same year or in the year following collection.
3.3.4 Breeding of strains with high yield and high artemisinin content

Although the cephaloid structure of the flowers is well suited for self-pollination, self-pollination is rather inefficient and fruiting is hampered by self-incompatibility (38). As a result, it is difficult to preserve the high-yielding characteristics of the strains by means of sexual propagation, and consequently the content of artemisinin among individual plants varies greatly. Therefore seed production bases should be established to continuously provide seeds of the germplasm with high yield of leaves and high content of artemisinin for large-scale production of raw materials.

3.4 Cultivation method

3.4.1 Selection and preparation of cultivation site

A favourable cultivation site should be chosen, preferably on a loose soil slope facing the sun. Plough, weed and level the field. Furrow and drain the field.

3.4.2 Sowing and nursery management

- **Sowing:** Seeds may be sown by scattering, in drills (rows) or in the spots where they are intended to grow.
- **Nursery management:** Care should be taken with irrigation of the nursery bed after budding, and seedlings should be thinned out after the 7th leaf appears. The quantity of seedlings required should be determined according to the cultivation conditions.

3.4.3 Fertilization

The base fertilizer should be applied before transplantation; the fertilizer used to facilitate branching should be added before the major branching stage, accompanied by weeding and hilling. The amount and type of fertilizer to be used should be determined according to the locality and cultivation conditions.

3.4.4 Field management

- **Drainage:** *A. annua* is sensitive to waterlogging, which can cause the roots to rot. In the rainy season, the channels and furrows should be cleared regularly.
- **Irrigation:** Throughout the whole process of growth and development of *A. annua*, irrigation and/or drainage should be carried out at times dependent upon the rainfall. In the case of long-term drought, watering should be carried out together with top dressing.
- **Weeding:** Manual weeding should be the main method of weeding. The use of chemical weedicides is forbidden. The first intertillage and scratch weeding should be done about 20 days after transplantation. The second weeding is needed before the major branching stage, followed by hilling. No intertillage weeding is necessary once the field is entirely covered by the plant. When necessary, only approved pesticides and herbicides should be applied at the minimum effective level, in accordance with the labelling and/or package
3.5 Prevention and control of plant diseases and pests

Root rot, virus disease and pests are the main diseases that occur during the growth period of *A. annua* in China. For different cultivation sites, the extent of plant disease and pest infestation should be evaluated to enable appropriate countermeasures to be taken, including selection of sites, and application of fertilizers and pesticides. Example are shown in Table 2 as a guide.

**Table 2: Examples of diseases and pests and their prevention and control**

<table>
<thead>
<tr>
<th>Typical diseases and pests</th>
<th>Main signs</th>
<th>Preventive and treatment measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root rot</td>
<td>Withering of the whole plant; root turns black and rots</td>
<td>Remove sick plants and apply treatment to site; crop rotation if appropriate</td>
</tr>
<tr>
<td>Viral diseases</td>
<td>New leaves appear wrinkled and shrunken; reduction in leaf size</td>
<td>Combined with aphid treatment, spray appropriate solutions on leaves</td>
</tr>
<tr>
<td>Aphids</td>
<td>Aphids in groups living on young leaves and undersides of leaves</td>
<td>Apply aphidicides</td>
</tr>
<tr>
<td>Ants</td>
<td>Attack plant at early growth stage; withering of top part of the plant, marks of gnawing on basal part of plant</td>
<td>Dig trenches around plants, place insecticide inside and cover</td>
</tr>
</tbody>
</table>

3.6 Harvest and postharvest processing

3.6.1 The best harvesting time, and quality evaluation prior to harvest

The range of artemisinin content of *A. annua* harvested from different production areas is wide. The highest content of artemisinin that can be reached is up to 1–2% (39) expressed as dry weight of leaves of *A. annua*. Although the content of artemisinin is affected by numerous factors such as geographical conditions, harvesting time, temperature and fertilizer application, harvesting at the appropriate time is critically important to ensure optimum content of artemisinin in *A. annua*. Therefore, the best time for harvesting of *A. annua* should be determined by a study of the weather conditions, dynamic accumulation and local harvesting experience. Studies in countries in which *A. annua* is produced have shown that the best harvesting time is the early stage of flower budding. The yield of *A. annua* leaves and the content of artemisinin is reduced if harvesting is too early or is delayed (40). The content of artemisinin of *A. annua* should be tested before harvesting.
3.6.2 Harvesting and post-harvest processing method

**Harvest**
On a sunny day during the harvesting season, cut down the whole crop of *A. annua*.

**Processing before drying**
*Aerial parts*. Remove foreign matter, spray with water, soften briefly, cut into sections (2).

**Drying**
- Dry in the sun (2).
- Different drying methods can affect the yield of artemisinin. Comparisons between sun-drying, shade-drying and oven-drying at 60 °C have shown that natural sun-drying is the best method (41).
- Protect from rain.
- When the leaves of *A. annua* have dried, strike or shake the wattle (branches) to detach the leaves from the stalks, and then remove the wattle, put the *A. annua* leaves into a sack or a lined mesh bag.

3.7 Personnel

Growers and producers should have an adequate knowledge of *A. annua*. This should include botanical identification, cultivation characteristics and environmental requirements, as well as the means of harvest and stage at which harvesting should be done. All personnel (including field workers) involved in the propagation, cultivation, harvest and postharvest processing stages of *A. annua* production should maintain appropriate personal hygiene and should have received training regarding their hygiene responsibilities. Only properly trained personnel, wearing appropriate protective clothing should apply agrochemicals. Growers and producers should receive instruction on all issues relevant to the protection of the environment, conservation of *A. annua* and proper agricultural stewardship.

For further information, see Annex 3. and refer to the WHO guidelines on good agricultural and collection practices for medicinal plants (1).
4. Good collection practices

The collection of medicinal plants raises a number of complex environmental and social issues that must be addressed locally on a case-by-case basis. The collection of wild *A. annua* should take into account the need to conserve wild resources, ensure correct species identification and take appropriate precautions for fieldwork. It is acknowledged that these issues vary widely from region to region and cannot be fully covered by these guidelines. More guidance can be found in the *WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants* (1), as well as *WHO/IUCN/WWF Guidelines on the conservation of medicinal plants* (42), which are currently being revised to deal comprehensively with the sustainable use and conservation of medicinal plants.

4.1. The principles for collecting wild *A. annua*

To legally collect wild resources, collection permits and other documents from government authorities of the countries in possession of *A. annua* must be obtained. As a basic principle, collection practices should not reduce the generative capacity of wild *A. annua*, and should not damage the natural habitat, to ensure sustainable utilization of wild plant resources. Therefore, before collection of *A. annua* begins, the reserves and population density of wild *A. annua* should be determined.

4.2 Permit for collecting

Before collection commences, the relevant legal and regulatory documentation issued by the country in which the *A. annua* is grown should be consulted. Collection can take place only when the appropriate procedure has been followed and permits have been obtained.

4.3 Botanical identification of wild *A. annua*

Local experts responsible for the field collection of *A. annua* should have received education and training in plant sciences and have practical experience in fieldwork. They should be able to distinguish the morphological characteristics of *A. annua* accurately. (See section 2.4 for a description of the morphological characteristics of *A. annua*). When necessary, the botanical identification of *A. annua* can be confirmed by a chemical method.
4.4 Requirements for collection

- Wild *A. annua* should not be collected in or near areas where high levels of pesticides or other possible contaminants occur, such as roadsides, drainage ditches, mine tailings, rubbish dumps and industrial facilities which may produce toxic emissions.
- In the course of collection, efforts should be made to remove any parts of the plant that are not required, and foreign matter, in particular toxic weeds. Decomposed *A. annua* should be discarded.
- Collecting implements, such as machetes, shears, saws and mechanical tools, should be kept clean and properly maintained. Those parts that come into direct contact with *A. annua* should be free from excess oil and other contaminants.
- If the collection site is located at some distance from the processing facilities, it may be necessary to sun-dry the *A. annua* prior to transport.
- While collecting *A. annua* in the field, all personnel must be protected from toxic and dermatitis-causing plants, poisonous animals and disease-carrying insects. Appropriate protective clothing, including gloves, should be worn when necessary.

4.5 Training of personnel prior to collection of plants

Local experts should be responsible for training the people who collect *A. annua*. They should be shown materials, pictures and other images of *A. annua* as part of their training. Local experts should also be responsible for the supervision of workers and the full documentation of the work performed. Field personnel should have adequate knowledge of *A. annua*, and be able to distinguish *A. annua* from similar plants. Collectors should also receive instruction on all issues relevant to the protection of the environment and the conservation of plant species, as well as the social benefits of sustainable collection of medicinal plants.

For further details, see annex 3 and the WHO guidelines on good agricultural and collection practice for medicinal plants (I).

4.6 Collecting time

The content of artemisinin is closely related to the time of collection. If not collected at the optimum time, the yield of *A. annua* leaves and the content of artemisinin will be affected (see section 3.6).

4.7 Quality evaluation prior to collection

Wild *A. annua* is widely distributed throughout the world. Confirmation that the expected quality of *A. annua* complies with that requested by the purchasers is vital (see section 3.6 and Chapter 5).
4.8 Others

For further details on collection methods, drying method, primary processing after drying and storage of herbal materials see section 3.6 and Chapter 6, respectively.
5. Quality control requirements for herbal materials of *A. annua*

5.1 Basic quality requirements for herbal materials

5.1.1 Selection of species or botanical variety

As a general principle, where applicable, the species or botanical variety selected for cultivation and/or for collection should be the same as that specified in the national pharmacopoeia or recommended by other authoritative national documents of the end-user's country, as the source for the herbal medicine concerned. In the absence of such national documents, the selection of species or botanical varieties specified in the pharmacopoeia or other authoritative documents of other countries should be considered. In the case of newly introduced medicinal plants, the species or botanical variety selected for cultivation and/or collection should be identified and documented as the source material used or described in the traditional medicine of the country of origin.

5.1.2 National quality specification and requirements for herbal materials

Usually a national pharmacopoeia specifies the official quality standard and provides analytical methods for quality control purposes. Currently, only two national pharmacopoeias in China and Viet Nam, include a monograph on *A. annua* (2, 43). National quality specifications on herbal materials of *A. annua* are required for quality control purposes. The following subsections describe quality specifications and analytical methods from the national pharmacopoeia to facilitate the establishment of a national quality specification in any country that wishes to do so. "Quality control methods for medicinal plant materials" also provides general analytical methods for establishing such specifications (44).

Unofficial standards established by suppliers and buyers vary. As this monograph focus on the herbal material of *A. annua*, intended for use as a raw material for the extraction of chemical constituents, the following basic quality specifications are for reference only. Countries may wish to set their own standard quality specification according to their own requirements and situation.

3 The titles of the monographs in the national pharmacopoeias are: "Herbal *Artemisia annua*" in the Chinese pharmacopoeia, and "Folium *Artemisia annua*" in the Vietnamese pharmacopoeia (2, 43)
5.2 Basic quality specification for herbal materials of A. annua

Organoleptic properties
Dried leaves of A. annua for extraction of artemisinin:

Colour: ranges from green to brown-green.
Odour: crushed leaves have a characteristic odour.
Taste: crushed leaves have a slightly bitter taste.

Water content:  
- aerial part: not more than 14% (2).
- leaves: not exceeding 13% (43).

Total ash:  
- aerial part: Not more than 8% (2)
- leaves: Not more than 6% (43).

Acid insoluble ash:  
- aerial part: Not more than 1% (2).

Ethanol-soluble extractives:  
- aerial part: not less than 1.9% using anhydrous ethanol as the solvent (2).

Branches and haulm:  
- leaves: not exceeding 10% (43).

Impurity:  
- leaves: not exceeding 2% (43).

Artemisinin content:  
- leaves: at least 0.7% (43).

Storage:  
Preserve in a cool dry place (2).

5.3 Methods for quality analysis

5.3.1 Determination of impurities\(^4\) of herbal materials (43)

Take a precisely weighed amount of raw material\(^5\) (P grams), scatter thinly on a piece of white paper, examine carefully by eye or using a magnifying glass. Separate the impurities, including dust and flour (sift through a flour sieve). Weigh the impurities (a grams).

The percentage of impurities (X%) is calculated as follows:

\[
X\% = \frac{a}{P} \times 100
\]

\(^4\) Impurities include all materials apart from the accepted standards for the raw material, i.e. soil, stones, rubbish, other herbs, other parts of the plants, and substandard products.

\(^5\) Quantity of test samples: very small seed and fruit, 10 g; small seed and fruit, 20 g; and sliced raw material, 50 g.
5.3.2 Test method for water content (2)

**Preparation of material**
The substance being examined is usually broken into granules or pieces of less than 3 mm in diameter. Materials which are already less than 3 mm in length and diameter can be examined without further breaking.

**Method**
Place in a flat weighing bottle, 2–5 g of the substance being examined which has previously dried to a constant weight, to form a smooth layer not exceeding 5 mm in thickness, or not exceeding 10 mm in thickness if the substance has a loose texture, and then weigh accurately. Dry in an oven at 100–105 °C for 5 hours with the stopper of the bottle removed. Upon opening the oven, close the bottle quickly and allow it to cool in a desiccator for 30 minutes. Weigh accurately and dry again under similar conditions for 1 hour, cool and weigh. Repeat the operation until the difference between two successive weighings is not more than 5 mg. Calculate the percentage content of water in the substance being examined according to the weight loss on drying.

5.3.3 Methods for chemical identification (2)

Take about 3 g of powder of dry leaves and add 50 ml of petroleum ether, heat under reflux for 1 hour (60–90 °C), filter, evaporate the filtrate to dryness, dissolve the residue in 30 ml n-hexane, extract 3 times with 10 ml 20% acetonitrile, mix the acetonitrile solution, evaporate the filtrate to dryness, dissolve the residue in 0.5 ml ethanol and use it as the test solution. Dissolve artemisinin in ethanol to produce a solution containing 1 mg/ml as the reference solution, in thin layer chromatography, using silica gel G as the coating substance and petroleum ether (60–90 °C)-ether (3:2) as the mobile phase. Apply separately to the plate 5 µl of each of the two solutions. After developing and removal of the plate, dry it in air, spray with 10% sulfuric acid–ethanol, heat at 105 °C for about 10 minutes. Examine under ultraviolet light (365 nm). The fluorescent spots in the chromatogram obtained with the test solution should correspond in position and colour to the fluorescent spots in the chromatogram obtained with the reference solution.

5.3.4 Methods for determination of artemisinin content (43)

**Preparation of test solution**
Take precisely 1 g of powder of dry leaves of *A. annua*, extract with petroleum ether (for analysis) in a 50 ml Soxhlet flask, heat in a bain marie until the whole content of artemisinin has been obtained. Evaporate the solvent, dissolve the residue with 1 ml chloroform (for analysis) and 9 ml ethanol 96° (for analysis) to prepare the test solution.

**Preparation of reference solution**
Take precisely 0.010 g of standard artemisinin, dissolve in 10 ml ethanol 96° (for analysis) to produce the reference solution.
Carry out the thin layer chromatography on a silica gel G plate (20 × 20 cm) activated at 110 °C/2 h, with 3 spots as follows:
   Spot 1: apply 0.1 ml of the test solution.
   Spot 2: apply 0.1 ml of the reference solution.
   Spot 3: apply 0.1 ml of the reference solution.
Use the solvent system toluene-ethyl acetate (95:5) as the mobile phase.

After developing and removal of the plate, dry it in air, cover spots 1 and 2; spray the area of spot 3 with the reagent PAB (0.25 g para-dimethylaminobenzaldehyde in 50 ml acetic acid and 5 ml of 10% phosphoric acid) to determine the site of artemisinin. Scrape the area of silica gel containing artemisinin of spots 1 and 2; scrape also a silica gel area that does not contain artemisinin as a white sample. Put in each of the 3 scraped silica gel powders 1 ml ethanol 96° (for analysis). Shake carefully. Add 9 ml sodium hydroxide (0.05 N), shake carefully, heat at 50 °C/30 min. Filter. Examine the filtrate under UV light (292 nm).

The percentage of artemisinin content in the absolutely dry leaves of *A. annua* is calculated as follows:

\[
\frac{Dt \times 100}{Dc \times P \times (100-B)}
\]

Dt: absorption band of the test solution  
Dc: absorption band of the reference solution  
P: mass of dry leaves of *A. annua* (g)  
B: moisture of the dry leaves of *A. annua* (%)
6. Other common management and technical requirements for both cultivated and collected *A. annua*

### 6.1 Bulk packaging

- Materials used for packaging should be non-polluting, clean, dry and undamaged and the outer materials should be resistant to tearing and should conform to the quality requirements for the medicinal plant materials concerned. Whenever possible, the packaging to be used should be agreed upon between supplier and buyer.
- Reusable packaging material such as sacks and lined mesh bags should be well cleaned (disinfected) and thoroughly dried prior to reuse, so as to avoid contamination by previous contents. All packaging materials should be stored in a clean and dry place that is free from pests and inaccessible to livestock, domestic animals and other sources of contamination.

### 6.2 Labelling

- The product name, specification, origin, batch number, packaging date and manufacturer should be indicated on each package of product.
- The label should also contain information indicating quality approval and should comply with other national and/or regional labelling requirements.
- To enable the product to be traced back to its origin, the batch number on the label should include information related to quality, cultivation date, date of harvesting or collection, producer, collector and processor.

### 6.3 Transportation

- Vehicles used for transporting bulk medicinal plant materials should be cleaned between loads. Bulk transport vehicles should be well ventilated to remove moisture from the dried leaves of *A. annua* and to prevent condensation.
- When pest infestations occur in bulk transport, fumigation should be carried out only when necessary; the fumigation equipment should be operated by licensed or trained personnel. Only registered chemical agents authorized by the regulatory authorities of the source country and the countries of intended end-use should be used.
6.4 Storage

- Where possible, the dried leaves of *A. annua* should be sent directly to a facility or factory for extraction of artemisinin.
- Owing to the existence of a special peroxide group in its chemical structure, artemisinin is unstable when heated. It decomposes readily if stored in conditions of high humidity and warm temperatures in the presence of a deoxidizing substance. The leaves should therefore not be stored at a high temperature.
- After harvesting or collection, the artemisinin content of the leaves decreases. Their value as a raw material for extraction of artemisinin would be lost after 1 year of storage (45).

6.4.1 Facilities and storage requirements

Storage facilities should be well aerated, dry and protected from light, and should be provided with humidity control equipment as well as facilities to protect against rodents and insects. The floor should be smooth, without cracks and easy to clean. Shelves on which to keep the materials should be built at a sufficient distance from the floor and the walls to prevent the occurrence of pest infestation, mould formation or rotting.

6.4.2 Time limit for storage

In general, 6 months is the maximum storage time for the harvested or collected leaves of *A. annua*. The artemisinin content should be tested when leaves are stored for longer than this period of time.

6.5 Quality assurance

Compliance with quality assurance measures should be verified through regular auditing visits to cultivation or collection sites and processing facilities by expert representatives of producers and buyers and through inspection by national and/or local regulatory authorities (1).

6.6 Document management

6.6.1 Documentation of production management

Standard operating procedures for handling *A. annua* should be adopted and documented. All processes and procedures involved in the production of *A. annua* and the dates on which they are carried out should be documented. The information should include date of operation and operator, all processes related to sowing, transplanting, fertilizing, pesticide use, irrigation and harvesting. All fumigation procedures, fumigation agents and dates of application should be documented.
6.6.2 Documentation of quality control management

All information related to quality control of *A. annua*, such as testing method, sample provider, testing agency, testing item, testing results, date of testing and signatures of authorized personnel should be documented.

6.6.3 Original records and others

All original records relating to *A. annua*, including the production plan, record of implementation, contracts and written agreements should be put on file and kept for at least 5 years. Files and archives should be maintained by specially designated personnel.

6.6.4 Export and import permits

For export of *A. annua* from the country of origin to another country, export permits, phytosanitary certificates, Convention on International Trade in Endangered Species (CITES) permit(s), and any other necessary permits must be obtained.

6.7 Personnel

All personnel (growers, collectors, producers, handlers, processors) should receive adequate training on relevant issues. Personnel issues including health, hygiene and sanitation are important. Details are given in Annex 3.
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Annex 1. List of participants in the WHO Consultation on Good Agricultural and Collection Practices for Artemisia annua L.

Nan Ning, Guanxi, China, 5–7 July 2005

Dr Yung-Hsien Chang, Vice President, China Medical University, Taiwan, China

Mrs Chen Xingyu, Director, Division of Cooperation, Department of International Cooperation, State Food and Drug Administration, Beijing, China

Mr Choi Peng Cheong, Chief, Division of Pharmacovigilance and Pharmacoeconomics, Department of Pharmaceutical Affairs, Health Bureau, Macau, Special Administrative Region, China [Co-Rapporteur: English]

Dr Daw Khin Phyu Phyu, Research Scientist, Biochemistry, Department of Medical Research (Upper Myanmar), Ministry of Health, Yangon, Myanmar [Co-Rapporteur: English]

Professor Ding Derong, College of Resource and Environment, South West Agriculture University, Chongqing, China

Dr Du Yingrong, Deputy Director, Department of Pharmaceutical and Medical Equipment, Beijing Municipal Health Bureau, Beijing, China [Co-Rapporteur: Chinese]

Mr Antony Ellman, Natural Resources Institute, Chatham, Kent, United Kingdom of Great Britain and Northern Ireland

Mr Guo Qingwu, Division of Drug Manufacturing Supervision, Department of Drug Safety and Inspection, State Food and Drug Administration, Beijing, China

Ms Han Peng, Program Officer, Division of Cooperation, Department of International Cooperation, State Food and Drug Administration, Beijing, China

Professor Huang Luqi, Director, WHO Collaborating Centre of Traditional Medicine, Institute of Chinese Materia Medica, China Academy of Traditional Chinese Medicine, Beijing, China

* It was a great sorrow to learn of the death of Professor Ding in August 2005. Her great contributions to WHO’s work, especially in the development of this GACP monograph on Artemisia annua L. will always be remembered.
Dr Mawuli Kofi-Tsekpo, Chief Research Officer, Kenya Medical Research Institute, Nairobi, Kenya [Co-Chairperson]

Mr Lan Yizhou, Director General, Guangxi Food and Drug Administration, Nan Ning, Guangxi, China

Ms Long Xi, Department of Crop Production, Ministry of Agriculture, Beijing, China

Ms Maria Noemia Marques Rodrigues, Chief, Department of Pharmaceutical Affairs, Health Bureau, Macau, Special Administrative Region, China

Dr Paulo Peter Mhame, Head, Department of Traditional Medicine Research, National Institute for Medical Research, Ministry of Health, Dar es Salaam, United Republic of Tanzania

Dr Chaturbhuja Nayak, Director, Central Council for Research in Homeopathy, Ministry of Health and Family Welfare, New Delhi, India

Professor Nguyen Gia Chan, Senior Researcher on Medicinal Plants, Hanoi, Viet Nam

Dr Nguyen Van Thuan, Director, Research Centre for Cultivation and Processing of Medicinal Plants, National Institute of Medicinal Materials, Hanoi, Viet Nam

Professor Qian Zhongzhi, Director, Traditional Chinese Medicine Division, State Pharmacopoeia Commission of China, Beijing, China [Co-Chairperson]

Dr Ren Dequan, Deputy Director-General, State Food and Drug Administration, Beijing, China

Professor Motoyoshi Satake, Institute of Environmental Science for Human Life, Ochanomizu University, Tokyo, Japan

Professor Setsuko Sekita, Department of Pharmacognosy and Natural Product Chemistry, Faculty of Pharmaceutical Sciences, Tokushima Bunri University at Kagawa Campus, Sanuki City, Kagawa, Japan

Ms Situ Sujian, Programme Officer, Division of International Organizations, Department of International Cooperation, Ministry of Health, Beijing, China

Dr Wang Naiping, President, Guangxi Traditional Chinese Medicine University, Nan Ning, Guangxi, China
Ms Wang Xiaona, Deputy Director, Department of Drug Safety and Inspection, Guangxi Food and Drug Administration, Nan Ning, Guangxi, China

Dr Wang Xiaopin, Deputy Director-General, Department of International Cooperation, State Administration of Traditional Chinese Medicine, Beijing, China

Mr Wang Zhexiong, Assistant Counsel, Department of Drug Safety and Inspection, State Food and Drug Administration, Beijing, China

Dr Weng Xinyu, Deputy Director, Division of Traditional Chinese Medicine, Department of Drug Registration, State Food and Drug Administration, Beijing, China [Co-Rapporteur: Chinese]

Professor Zhang Bengang, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing, China

Dr Zhong Guoyue, Chongqing Academy of Chinese Materia Medica, Chongqing, China

Ms Zhang Jing, Technical Consultant, Department of Pharmaceutical Affairs, Health Bureau, Macau, Special Administrative Region, China

Dr Zou Jianqiang, Director, Biotech and Health Division, Department of Rural Science and Technology, Ministry of Science and Technology, Beijing, China

Representatives of other UN organizations and offices

FAO (Food and Agriculture Organization of the United Nations)
Mr Peter Griffee, Senior Officer, Industrial Crops, FAO, Rome, Italy

UNICEF (United Nations Children's Fund)
Mr Song Xiaobing, Supply Officer, Supply Division, UNICEF, Beijing, China

Local Secretariat

Mr Chen Yehui, Director, General Office, Guangxi Food and Drug Administration, Nan Ning, Guangxi, China

Mr Yun Liao, Secretary, General Office, Guangxi Food and Drug Administration, Nan Ning, Guangxi, China
**WHO Secretariat**

Dr Ossy Kasilo, Regional Adviser, Traditional Medicine, WHO Regional Office for Africa, Brazzaville, Congo

Ms Yukiko Maruyama, Scientist, Traditional Medicine, Department of Technical Cooperation for Essential Drugs and Traditional Medicine, WHO, Geneva, Switzerland

Dr Allan Schapira, Coordinator, Strategy and Policy team (MSP), Roll Back Malaria Department, WHO, Geneva, Switzerland

Dr Xiaorui Zhang, Coordinator, Traditional Medicine, Department of Technical Cooperation for Essential Drugs and Traditional Medicine, WHO, Geneva, Switzerland
Annex 2. Quality specification for artemisinin and its chemical derivatives

Reproduced from International pharmacopoeia, 3rd ed, Vol. 5 (artemisinin, pp 198–202; artemether, pp 187–190; artesunate, pp 222–225). The page numbers and section numbers indicated in the following description refer to those that appear in the original print copy of the International pharmacopoeia. For further technical details, please refer to the relevant section of the International pharmacopoeia. Because the International pharmacopoeia is constantly being updated, the most recent technical information should be obtained by consulting the latest edition of the International pharmacopoeia.

Artemisininum - Artemisinin

\[ \text{C}_{15}\text{H}_{22}\text{O}_5 \]

Relative molecular mass. 282.3

Chemical name. (3R,5aS,6R,8aS,9R,12S,12aR)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4.3-j]-1,2-benzodioxepin-10(3H)-one; CAS Reg. No. 63968-64-9.

Description. Colourless needles or a white, crystalline powder.

Solubility. Practically insoluble in water; very soluble in dichloromethane R; freely soluble in acetone R and ethyl acetate R; soluble in glacial acetic acid R, methanol R and ethanol (~750 g/l) TS.

Category. Antimalarial drug.

Storage. Artemisinin should be kept in a well-closed container, protected from light and stored in a cool place.
Requirements

Artemisinin contains not less than 97.0% and not more than the equivalent of 102.0% of C₁₅H₂₂O₅ using Assay method A, and not less than 98.0% and not more than the equivalent of 102.0% of C₁₅H₂₂O₅ using Assay method B, both calculated with reference to the dried substance.

Identity tests

• Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from artemisinin RS or with the reference spectrum of artemisinin.

B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. Dissolve 5 mg in about 0.5 ml of dehydrated ethanol R, add about 0.5 ml of hydroxyamine hydrochloride TS2 and 0.25 ml of sodium hydroxide (~80 g/l) TS. Heat the mixture in a water-bath to boiling, cool, add 2 drops of hydrochloric acid (~70 g/l) TS and 2 drops of ferric chloride (50 g/l) TS; a deep violet colour is immediately produced.

D. Dissolve 5 mg in about 0.5 ml of dehydrated ethanol R, add 1.0 ml of potassium iodide (80 g/l) TS, 2.5 ml of sulfuric acid (~100 g/l) TS and 4 drops of starch TS; a violet colour is immediately produced.

Melting range. 151–154 °C.

Specific optical rotation. Use a 10 mg/ml solution in dehydrated ethanol R;

\[ \alpha_D^{20} = +75° \text{ to } +78° \]

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 80 °C; it loses not more than 5.0 mg/g.

Related substances

• Either test A or test B may be applied.

A. Carry out the test as described under "High-performance liquid chromatography" (p. 257), using a stainless steel column (10 cm × 4.6 mm) packed with stationary phase A (µm). The mobile phases for gradient elution consist of a mixture of acetonitrile and water, using the conditions shown in the following table:
Prepare the following solutions. For solution (A) use 10 mg of Artemisinin per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water, and for solution (B) use 50 µg of Artemisinin per ml in a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

For the system suitability test prepare solution (c) containing 1 mg of artemisinin RS per ml and 1 mg of artemimol RS per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A, B and C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution B (0.5%). Not more than one peak is greater than half the area of the principal peak obtained with solution B (0.25%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution B (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution B. The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

B. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R1 as the coating substance and a mixture of equal volumes of light petroleum R1 and ether R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in toluene R containing (A) 10 mg of Artemisinin per ml, (B) 0.05 mg of Artemisinin per ml, (C) 0.025 mg of Artemisinin per ml, (D) 0.10 mg of Artemisinin per ml, and (E) 0.10 mg of artemisinin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with anisaldehyde/ sulfuric acid TS, and heat the plate to 105 °C for 7 minutes. Examine the chromatogram in daylight.
Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

**Assay**

- *Either method A or method B may be applied.*

A. Determine by "High-performance liquid chromatography" (p. 257), using a stainless steel column (10 cm × 4.6 mm) packed with *stationary phase A* (3 µm). As the mobile phase, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

Prepare the following solutions in the mobile phase: solution (A) 1.0 mg of Artemisinin per ml; and solution (B) 1.0 mg of artemisinin RS per ml.

For the system suitability test prepare solution (c) containing 1 mg of artemisinin RS per ml and 1 mg of artemimol RS per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A, B and C.

The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of C₁₅H₂₂O₅ with reference to the dried substance.

B. Dissolve about 0.05 g of Artemisinin, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml, and dilute 10 ml to 100 ml with the same solvent. Accurately transfer 10 ml to a 50-ml volumetric flask, dilute to volume with sodium hydroxide (0.05 mol/l) VS, mix thoroughly, and warm to 50 °C in a water-bath for 30 minutes. Cool to room temperature.

Measure the absorbance of a 1-cm layer at the maximum at about 292 nm against a solvent cell containing a blank prepared with 10 ml of ethanol (~750 g/l) TS diluted with sufficient sodium hydroxide (0.05 mol/l) VS to produce 50 ml. Calculate the percentage content of C₁₅H₂₂O₅ in the substance being tested by comparison with artemisinin RS, similarly and concurrently examined, and with reference to the dried substance.
Artemetherum - Artemether

\[ \text{C}_{16}\text{H}_{26}\text{O}_{5} \]

Relative molecular mass. 298.4

Chemical name. (3R,5aS,6R,8aS,9R,10S,12R,12aR)-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxeprin; CAS Reg. No. 71963-77-4.

Description. White crystals or a white, crystalline powder.

Solubility. Practically insoluble in water; very soluble in dichloromethane R and acetone R; freely soluble in ethyl acetate R and dehydrated ethanol R.

Category. Antimalarial drug.

Storage. Artemether should be kept in a tightly closed container, protected from light and stored in a cool place.

Labelling. The designation Artemether for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration.

Additional information. The parenteral form is normally intended for intramuscular administration.

Requirements

Artemether contains not less than 97.0% and not more than the equivalent of 102.0% of C\(_{16}\)H\(_{26}\)O\(_{5}\) using Assay method A, and not less than 98.0% and not more than the equivalent of 102.0% of C\(_{16}\)H\(_{26}\)O\(_{5}\) using Assay method B, both calculated with reference to the dried substance.

Identity tests

- Either tests A and B or tests B, C, and D may be applied.

A. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from artemether RS or with the reference spectrum of artemether.
B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. To 30 mg add about 1 ml of dehydrated ethanol R and about 0.1 g of potassium iodide R. Heat the mixture on a water-bath; a yellow colour is produced.

D. Dissolve 30 mg in 6.0 ml of dehydrated ethanol R. Place a few drops of the mixture on a white porcelain dish and add 1 drop of vanillin/sulfuric acid TS1; a pink colour is produced.

**Melting range.** 86.0–90.0 °C.

**Specific optical rotation.** Use a 10 mg/ml solution in dehydrated ethanol R;

\[ [\alpha]_D^{20^\circ} = +166^\circ \text{ to } +173^\circ. \]

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry over phosphorus pentoxide R under reduced pressure (not exceeding 2.67 kPa or 20 mm of mercury); it loses not more than 5.0 mg/g.

**Related substances**

- **Either test A or test B may be applied.**

A. Carry out the test as described under "High-performance liquid chromatography" (p. 257), using the conditions given below under Assay method A.

Inject alternately 20 µl each of solutions A and C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage.

In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution C (0.5%). Not more than one peak is greater than half the area of the principal peak obtained with solution C (0.25%). The sum of the areas of all the peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution C (1.0%). Disregard any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with solution C.

B. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R1 as the coating substance and a mixture of 7 volumes of light petroleum R1 and 3 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in acetone R containing (A) 10 mg of Artemether per ml, (B) 0.05 mg of Artemether per ml, (C) 0.025 mg of Artemether per ml, (D) 0.10 mg of Artemether per ml, and (E) 0.10 mg of artemether RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/sulfuric acid TS1. Examine the chromatogram in daylight.
Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

**Assay**

* Either method A or method B may be applied.

A. Determine by "High performance liquid chromatography" (p. 257), using a stainless steel column (25cm × 4mm) packed with stationary phase A (5 µm). As the mobile phase, use a mixture of 62 volumes of acetonitrile R and 38 volumes of water.

Prepare the following solutions in the mobile phase: solution (A) 10 mg of Artemether per ml; solution (B) 10 mg of artemether RS per ml; and for solution (C) dilute solution A to obtain a concentration equivalent to 0.05 mg of Artemether per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of C₁₆H₂₆O₅ with reference to the dried substance.

B. Dissolve about 0.050 g of Artemether, accurately weighed, in sufficient dehydrated ethanol R to produce 100 ml. Dilute 2 ml of this solution to 100 ml with hydrochloric acid/ethanol (1 mol/l) VS. Stopper the flask and place it in a water-bath at 55 °C for 5 hours. Allow to cool to room temperature.

Measure the absorbance of this solution in a 1-cm layer at the maximum at about 254 nm. Calculate the percentage content of C₁₆H₂₆O₅ by comparison with artemether RS, similarly and concurrently examined, and with reference to the dried substance.

**Additional requirement for Artemether for parenteral use**

Complies with the monographs for "Parenteral preparations" (see Vol. 4, p. 36), "Test for extractable volume for parenteral preparations" (see p. 27), and "Visual inspection of particulate matter in injectable preparations" (see p. 33).
Artesunatum - Artesunate

\[
C_{19}H_{28}O_8
\]

Relative molecular mass. 384.4

Chemical name. \((3R,5aS,6R,8aS,9R,10S,12R,12aR)\)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12\(H\)-pyran-[4,3-\(j\)]-1,2-benzodioxepin-10-ol, hydrogen succinate; CAS Reg. No. 88495-63-0.

Description. A fine, white crystalline powder.

Solubility. Very slightly soluble in water; very soluble in dichloromethane \(R\); freely soluble in ethanol (\(~750 \text{ g/l}\) \(TS\)) and acetone \(R\).

Category. Antimalarial drug.

Storage. Artesunate should be kept in a well-closed container, protected from light and stored in a cool place.

Requirements

Artesunate contains not less than \(96.0\%\) and not more than the equivalent of \(102.0\%\) of \(C_{19}H_{28}O_8\) using Assay method A, and not less than \(99.0\%\) and not more than the equivalent of \(101.0\%\) of \(C_{19}H_{28}O_8\) using Assay method B, both calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from artesunate \(RS\) or with the reference spectrum of artesunate.

B. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel \(R6\) as the coating substance and a mixture of 5 volumes of ethyl acetate \(R\) and 95 volumes of toluene \(R\) as the mobile phase. Apply separately to the plate 2\(\mu\)l of the following 2 solutions in toluene \(R\) containing (A) 0.10 mg of Artesunate per ml, and (B) 0.10 mg of artesunate \(RS\) per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with anisaldehyde/methanol \(TS\),...
and heat the plate to 120 °C for 5 minutes. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve 0.1 g of Artesunate in 40 ml of dehydrated ethanol R, shake, and filter. To half of the filtrate (keep the remaining filtrate for test D) add about 0.5 ml of hydroxylamine hydrochloride TS2 and 0.25 ml of sodium hydroxide (~80 g/l) TS. Heat the mixture in a water-bath to boiling, cool, add 2 drops of hydrochloric acid (~70 g/l) TS and 2 drops of ferric chloride (50 g/l) TS; a light red-violet colour is produced.

D. Evaporate the remaining filtrate from test C on a water-bath to a volume of about 5 ml. Place a few drops of the mixture on a white porcelain dish, add 1 drop of vanillin/sulfuric acid TS2, and allow to stand for 30 minutes; a red colour is produced.

**Melting range.** 132–135 °C.

**Specific optical rotation.** Use a 10 mg/ml solution in dichloromethane R;

\[
\left[\alpha\right]_{D}^{20} = +2.5^\circ \text{ to } +3.5^\circ.
\]

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under "Limit test for heavy metals", Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 20 µg/g.

**Sulfated ash.** Not more than 1.0 mg/g.

**Water.** Determine as described under "Determination of water by the Karl Fischer Method", Method A (Vol. 1, p. 135), using 2 g of Artesunate; the water content is not more than 5 mg/g.

**pH value.** pH of an aqueous suspension containing 10 mg/g, 3.5–4.5.

**Related substances**

- *Either test A or test B may be applied.*

A. Carry out the test as described under "High-performance liquid chromatography" (p. 257), using the conditions given below under Assay method A.

Inject alternately 20 µl each of solutions A and C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution C (1.0%). Not more than one peak is greater than half the area of the principal peak obtained with solution C (0.5%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution C (2.0%). Disregard
WHO monograph on good agricultural and collection practices (GACP) for Artemisia annua L.

any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution C.

B. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R1 as the coating substance and a mixture of 48 volumes of light petroleum R1, 36 volumes of ethyl acetate R and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of the following 3 solutions in dichloromethane R containing (A) 5.0 mg of Artesunate per ml, (B) 0.05 mg of Artesunate per ml, and (C) 0.025 mg of Artesunate per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/sulfuric acid TS1. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (1.0%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%).

Assay

- *Either method A or method B may be applied.*

A. Determine by "High-performance liquid chromatography" (p. 257), using a stainless steel column (12.5 cm × 3.5 mm) packed with stationary phase A (5 µm). As the mobile phase, use a mixture of equal volumes of acetonitrile R and buffer pH 3.0 (dissolve 1.36 g of potassium dihydrogen phosphate R in 1000 ml of water and adjust the pH to 3.0 with phosphoric acid (~1440 g/l) TS).

Prepare the following solutions in acetonitrile R: solution (A) 4.0 mg of Artesunate per ml; solution (B) 4.0 mg of artesunate RS per ml; and for solution (C) dilute solution A to obtain a concentration equivalent to 0.04 mg of Artesunate per ml.

Operate with a flow rate of 0.6 ml per minute. Maintain the column temperature at 30 °C and use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of C_{19}H_{28}O_{8} with reference to the anhydrous substance.

B. Dissolve about 0.25 g of Artesunate, accurately weighed, in 25 ml of neutralized ethanol TS and titrate with sodium hydroxide (0.05 mol/l) VS, using 2 drops of phenolphthalein/ethanol TS as indicator.

Each ml of sodium hydroxide (0.05 mol/l) VS is equivalent to 19.22 mg of C_{19}H_{28}O_{8}.  

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Annex 3. Personnel

Reproduced from WHO guidelines on good agricultural and collection practices for medicinal plants (1), section 4.7, and reproduces original heading numbers.

4.7.1 General

All personnel should receive adequate botanical and agricultural or collection training.

All personnel required to apply agrochemicals should be trained in their use. Producers and collectors should receive adequate training and possess sufficient knowledge about appropriate harvesting and techniques employed for plant maintenance and protection for the medicinal plants to be cultivated.

To avoid deterioration of harvested medicinal plant materials during the post-harvest handling and primary processing stages, proper training of all personnel involved is required.

Personnel should be instructed on all relevant issues regarding environmental protection, the conservation of plant species and proper soil management to conserve fields for cultivation and for soil erosion control. The prevention of environmental degradation is an essential requirement to ensure the sustainable long-term use of medicinal plant resources.

National and/or regional regulations governing labour should be respected in the employment of staff for all phases of medicinal plant materials production.

4.7.2 Health, hygiene and sanitation

All production of medicinal plant materials by agriculture and collection should conform to national and/or regional regulations on safety, materials handling, sanitation and hygiene.

All those involved in the handling and processing of cultivated or collected medicinal plants should in all processing procedures comply with national and/or regional regulations on hygiene.
All personnel should be protected from contact with toxic or potentially allergenic herbs by means of adequate protective clothing, including gloves.

**Health status**
No personnel known, or suspected, to be suffering from or to be a carrier of a disease or illness likely to be transmitted through medicinal plant material, should be allowed to enter any harvest, production or processing area if there is a likelihood of their contaminating medicinal plant materials. Any persons suffering from diseases or symptoms of illness should immediately report to the management. A medical examination of personnel should be carried out if clinically or epidemiologically indicated.

**Illness and injuries**
All personnel with open wounds, inflammations or skin diseases should be suspended from work or required to wear protective clothing and gloves until fully recovered. Persons suffering from known airborne or food-borne communicable diseases, including dysentery and diarrhoea, should be suspended from work in all areas of production and processing, in accordance with local and/or national regulations.

Health conditions that should be reported to the management for consideration regarding medical examination and/or possible exclusion from handling of medicinal plant materials include: jaundice, diarrhoea, vomiting, fever, sore throat with fever, visibly infected lesions (e.g. boils and cuts) and discharges from the ear, nose or eye. Any personnel who have cuts or wounds and are permitted to continue working should cover their injuries with suitable waterproof dressings.

**Personal cleanliness**
Personnel who handle medicinal plant materials should maintain a high degree of personal cleanliness, and, where appropriate, wear suitable protective clothing and gloves, including head covering and footwear.

Personnel should always wash their hands at the start of handling activities, after using the toilet, and after handling medicinal plant materials or any contaminated material.

**Personal behaviour**
Smoking and eating should not be permitted in medicinal plant processing areas.
Personnel who handle medicinal plant materials should refrain from behaviours that could result in contamination of the materials, for example, spitting, sneezing or coughing over unprotected materials.

Personal effects such as jewellery, watches or other items should not be worn or brought into areas where medicinal plant materials are handled if they pose a threat to the safety or quality of the materials.
**Visitors**
Visitors to processing and handling areas should wear appropriate protective clothing and adhere to all of the personal hygiene provisions mentioned above.