Original Article

Artemisia annua L., Potential Source of Molecules with Pharmacological Activity in Human Diseases

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ABSTRACT

Objective: This review intends to motivate and encourage researchers to explore new alternatives to treat different diseases with *Artemisia annua* L., an important plant of traditional Chinese pharmacopoeia that has been used for more than 2.000 years in the treatment of different diseases, mainly malaria.
Methods: Data include currently available information about *A. annua*, its origin, traditional use in medicine, pharmacological activity, toxicity and main metabolites with reported clinical activity. The information was collected by literature search on web databases such as Pubmed and Google Scholar up to 2014 on publications about the medicinal uses of *A. annua* L., in the treatment of different diseases that affect humans but also some animals.
Results: Pharmacological activity against chronic and infectious diseases of various metabolites from *A. annua*, artemisinin and its derivatives, flavonoids and essential oils, reported in this review, is supported by preclinical experimental evidence both *in vivo* and *in vitro* and clinical observations in human beings of different parts of the plant, mainly leaves, in the treatment of malaria. Leaves, seeds and whole plant of *A. annua* have also proved pharmacological activity against parasites responsible of leishmaniasis and Chagas disease. Recently, the first report of *in vivo* efficacy of *A. annua* against dengue fever was published.
Conclusions: This review highlights the pharmacological potential of the *A. annua* plant in the treatment of several infectious diseases and unveils its suitable profile of safety and tolerability.

Keywords: *Artemisia annua*, Antibacterial activity, Antiparasitic activity, Anti-inflammatory activity, Antiviral potential, Anthelmintic activity.

INTRODUCTION

Artemisia annua L., is a plant included in the Chinese Pharmacopeia that has been used since more than 2000 years to cure a broad array of diseases. During the Vietnam war the whole plant was supplied to the Vietcong as a remedy against malaria. In 1972 a Chinese research team discovered in the leaves of the plant a molecule, which they called artemisinin or quinhaosu. This molecule and its derivatives were initially used in monotherapy against malaria. But after 30 years of use severe resistance developed. WHO recommended to combine artemisinin with other long lasting antimalarials like lumefantrine, mefloquine or amodiaquine. These combinations are called ACT (artemisin combined therapy). The discovery of the plant *A. annua* was a major breakthrough in the fight against malaria.

There are however some problems relating to the use of artemisinin. The main problem is that it is a very fast acting drug with a very short half-life. It therefore needs to be combined with a slower acting drug in order to assure the effective elimination of all the parasitaemia. Another problem is the assurance of an adequate supply of artemisinin. Due to its complex chemical structure the synthesis and/or semi-synthesis remains to be difficult and therefore the main source of artemisinin remains to be isolation and purification from the *A. annua* plant. In addition, the annual production of *A. annua* varies from a boom and bust cycle with overproduction and low prices the one year to underproduction and high prices the next year due to environmental and economic influences. The cost of this Western style treatment is also far too high for where it is used, predominantly in non-Western countries[1].

Scientific name

Artemisia annua L. (Latin name) belongs to Asteraceae Family (also known as Compositae), Genus: *Artemisia* and Especie: *A. annua.* Carl von Linnaeus already listed the plant in his *Species Plantarum* in 1793.

Common name

A. annua is commonly known in English as “sweet wormwood”, “annual wormwood”, “sweet sagewort” or “sweet annie”. In Chinese is named “qinghao” or “huag hua hao” while in French is called “armoise annuelle”.

Origin

A. annua is an annual plant of Chinese origin. It spread in Europe and America in river valleys as offspring of seeds imported by merchant vessels but it grows preferably at altitudes of 1000 to 1500 m. It has a vegetation period of six months. Its leaves are extensively used in Chinese Traditional Medicine (TCM) for the treatment of malaria, fever, tuberculosis, nematodes, ulcers, diarrhea[1].

Distribution

Currently, *A. annua* grows in all continents and climates. The plant however prefers countries with pronounced summers and winters and does not like equatorial days with a constant daylight duration of 12 hours. It is cultivated at large scale in Asia and Africa for the extraction and supply of artemisinin. Other countries are adopting their own plantations to promote the use of this plant in the traditional medicine[2].

Constituents of *A. annua* L. of pharmacological use or interest

The best known of these constituents is the sesquiterpenoid artemisinin and its chemical derivatives artesunate, artemether,

arteether. The plant not only has a high content of polyphenols, flavonoids, proteins, coumarins, phytosterols, polysaccharides but also in potassium, selenium and nitrate inorganic salts. Volatile essential oils are present at concentrations of 0.20-0.25%. Other important constituents include camphene, ketone, camphor, beta-caryophyllene, pinene and 1, 8-cineole[2].

Artemisinin

Artemisin is a sesquipterpene lactone containing an endoperoxyde bridge that is present in many other species of *Artemisia*[3], although at very low concentrations. *In vitro* artemisinin has one of the lowest LD_{50} against *Plasmodium* spp. *In vivo* it is also very active against various stages of *Plasmodium* of different species. The toxicity of the molecule is negligable at therapeutic doses; however, repeated use at high doses of artemisinin has been associated with haemolysis and toxicities in liver, spleen, nervous and cardiovascular system; artemisinin is also considered genotoxic, ototoxic and embryotoxic[4].

Artemisinin derivatives

Artemisinin has been chemically modified by pharmaceutical companies to enhance its solubility either in water (artesuante) or in lipids (artemether). It is claimed that these derivatives are 5 times more efficient than artemisinin although scientific data to substantiate this claim are scarce. They are rapidly metabolized into dihydroartemisinin and their half live in the human body is very short. One of the first mechanisms which was proposed for the action of the artemisinins was that they interfered with digestion of hemoglobin in the food vacuole. This theory is based on the Fenton reaction in which the endoperoxide reacts with iron and creates a flurry of radicals which kill the parasite. Other studies indicate that the artemisinins attack the mitochondria of the parasite, and more specifically the protein TCTP or the PfATP6, an ATP localized in the sarcoplasma of the parasite[5]. More recent studies with yeast indicate that artemisinins change the potential of the mitochondrial membrane if the glycolysis of ethanol, glycerol and other sugars are inhibited.

There are several other approaches trying to explain the action of artemisin. However, after three decades these theories are still conflicting and how artemisinins work remains to be understood[5].

Flavonoids

Over the last 40 years hundreds of peer reviewed papers have concentrated their research on the antiparasitic effect of artemisinin and its chemical derivatives while other constituents have deserved less attention. Particularly polyphenols and flavonoids are now considered as key actors in the efficiency of *A. annua* and other plants of this family. Flavonoids such as artemetin, rutin, quercetin, casticin, eupatin, luteolin and their glucosides have a variety of biological activities many of them synergizing the effects of artemisinin. Luteolin has shown antimalarial and antioxidant activities[6]. In general flavonoids inhibit CYP3A4 which is responsable for the rapid metabolism of artemisinin. Flavonoids are known to persist in the body for > 5 days; this may explain that a once a week dose induces a prophylactic effect from *A annua* tea infusion[7,8].

Coumarins

A. annua contains several coumarins and most *Artemisia* species contain predominantly scopoletin which is considered hepatoprotective. Scopoletin has also immunomodulatory and anti-inflammatory activities[9]. The concentration of scopoletin in several samples of *A. annua* as measured at Luxembourg is around 0.2%

(w/w). Coumarins have anti-coagulatory properties[10,11].

Essential oils

The major constituents of the essential oils in *A. annua* are cineole, camphene, a-pinene, germacrene,, camphor and ketone. They are present at concentrations of 0.20-0.25% and have shown not only different antimicrobial activities[12] but also anti-inflammatory[13] and cytotoxic activities[14,15]. They are drastically reduced by high drying temperatures of the herb. Artemisia ketone is a major constituent of some cultivars of *A. annua*. Like other ketones it inhibits hemozoin crystallization in malaria infected persons. Nerolidol has an IC_{50} of 0.99 µmol/L and may lead to 100% growth inhibition at the schizont stage.

Phenolic acids

Phenolic acids chlorogenic and rosmarinic are present in tea infusion of *A. annua*. They are strong antioxidants and may reduce secretion of anti-inflammatory cytokines IL-6 and IL-8. They are present in many especies of *Artemisia*[16].

Polysaccharides and saponins

These molecules seem to have been ignored in *A.annua*, probably because the organic solvents used during extractions and polysaccharides are only soluble in water. Polysacharides of *A. annua* have been tested as adjuvants in the hepatitis C vaccine, showing that these polysaccharides may promote IFN-γ production[17]. The combination of polysaccharides with lipophilic molecules like artemisinin may lead to a higher bioavailability. Sulfated polysaccharides inhibit blood-stage growth of plasmodium[18]. They also inhibit the formation of rosettes between infected erythrocytes. Chinese manufacturers of capsules add the polysaccharide inulin to the *A. annua* powder.

In turn, saponins, common in many plants, have been described in *A. abrotanum*[19] and *A. sphaerocephala*[20]. They play an important role in human and animal nutrition. At very low doses saponins have a hypoglycemic effect, hemolytic properties and modulate the sodium pumps and ATPase[21].

Phytosterols

The *Artemisia* genus contains around 200 mg of phytosterols per 100 g of dry matter[22]. The fact that phytosterols have been neglected in the chromatographic analysis of *A. annua* and other medicinal plants is because they are difficult to analyze.

Inorganic constituents

Artemisia species are high in inorganic constituents such as selenium, iron, gallium, potassium, carbonates and nitrates. However to date there are no studies describing exactly the amounts of these constituents.

- #### Potassium

The *Artemisia* genus have the highest potassium content among all medicinal plants[23]. Potassium concentrations in *A. annua* are 10 to 100 times higher than those of other minerals, particularly sodium. A more complete study in Pakistan, comparing 10 medicinal plants finds that potassium content in *A. annua* is the highest (P. Lutgen, personal communication).

- #### Selenium

Apparently, species of *Artemisia* genus accumulate many minerals, including selenium, several times more than fruits and vegetables[24,25]. Because selenium is an important element for immunity, these may explain the beneficial effect of the *A. annua* on the immune system[26].

- Gallium

Many plants of the *Artemisia* family are accumulators of metals and heavy metals[27], however, no information about the specific amounts of these metals in Artemisia are available.

- Bicarbonate

Plants may contain millimolar concentrations of bicarbonate. The uptake of this salt is positively correlated with its presence in the soil and it is much more concentrated in stems than in leaves[28]. Sodium bicarbonate added to *A. annua* or *A. sieberi* infusion increased the inhibition of beta-hematin crystallization (Akkawi, University of Al Quds, Palestine, unpublished results).

- Nitrate

Many anecdotical or scientific results indicate that leaves and stems of *A. annua* have different therapeutical properties, often higher for leaves, sometimes lower. There appears to be one major difference betweeen stems (stalks, twigs, petioles) and leaves: a 2-3x lower concentration of nitrate in leaves[29].

Therapeutic potential of *A. annua* L. derivatives in humans

Malaria

Artemisinin is probably one of the major constituents responsable for the antimalarial properties of *A. annua*. Although other plants of this family, which do not contain artemisinin, like *A. absinthium, A. herba alba, A. apiacea, A. ludoviciana, A. abrotanum* and particularly *A. afra* have excellent antimalarial properties. The latter is widely used for this application in South-Africa and Tanzania.

The association IFBV-BELHERB from Luxembourg is actively promoting these other varieties, because the resistance to artemisinin monotherapy which has emerged already 20 years ago, now also affects the ACTs (Coartem®, Coarsucam®) not only in South East Asia, but has spread to eight African countries. IFBV-BELHERB with partners at African universities[30] or in cooperation with Ministries of Health in these countries has run several clinical trials which document the effectiveness of the whole plant, also knwn as "totum" served as infusion in hot water encapsulated powdered leaves, tablets (also named pACTs) or simply as powder mixed with peanut butter or porridge. A dose of 24 mg/kg of Artemisia powder was much more effective in reducing parasitemia in mice infected with *Plasmodium chabaudi* than an equivalent dose of pure artemisinin[31]. More recently the same research team at the Worcester Institute reports that the consumption of dried whole-plant *A. annua* delays the appearance of malaria drug resistance and overcomes resistance to artemisinin[32]. This is a very important finding in the fight against malaria and the resistance to monotherpies. The authors relate this to the synergistic action of artemisinin with other molecules present in the plant[33].

The Université des Montagnes in Cameroon has developed a form more suitable for small children who don't like the bitterness of the tea[34]. But more important even is the fact that many people consuming regularly *A.annua* whole leaf in one form or another have noticed a prophylactic effect[7,8]. By reducing the frequency of malaria infections in a given population someday the threshold will be reached like in Europe and North America where the number of malaria infected people, symptomatic or asymptomatic, is so low that the transmission chain from mosquito to man is broken.

Leishmaniasis

Cutaneous leishmaniasis (CL) is endemic in at least 98 countries worldwide. The currently available drugs are costly, used under long treatment schemes and are becoming less effective. Therefore, discovery of new drugs is needed. Recently, the hámster model for CL was optimized by inoculation in the dorsal skin. This approach demonstrated that the clinic and pathologic features of CL are remarkably similar to the human disease. Leaves and seeds of *A. annua* were effective in *in vitro* and *in vivo* trials in male and female golden hamster. Animals were treated after typical skin lesions had developed. Compound was administered via oral once per day during 20 days. The effectiveness of the treatment was determined by comparison of the lesion size before and after treatment. Complete cure began observed after one month of the end of treatment. After 3 months of the end of treatment, the therapeutic response was 100% in the group of hamsters treated with the highest dose[35,36]. These results suggest that tea from *A. annua* is highly effective against Leishmania parasites and could be a promising candidate for oral treatment for CL. An Indian *in vitro* study made with leaves and seeds of *A. annua* confirmed this efficiency against amastigotes and promastigotes de *L. donovani*. No cytotoxic effect was noticed[37].

Anthelmintic and anti-trematode effects

A. annua is commonly named "wormwood" because its anthelmintic properties are known since ages. It is mentioned in the Bible (Revelation 8:10). One of the partners of IFBV-BELHERB in Senegal has noticed that *A. annua* is efficient in the treatment of schistosomiasis (bilharziosis) which is the second most important disease in tropical Africa. Clinical trials are underway to confirm this finding.

Trematode infections negatively affect human and livestock health, and threaten global food safety. Only triclabendazole and praziquantel are the drugs approved as human anthelmintics for trematodiasis.. Crude extracts of *A. annua, Asimina triloba, a*nd *A. absinthium* were tested against adult *Schistosoma mansoni, Fasciola hepatica,* and *Echinostoma caproni in vitro*. The ethanolic extract were more active[38].

Chagas' disease (*Trypanosoma cruzi*)

It has been demonstrated that low doses of artemisinin are able to inhibit the development of *T. cruzi* and *T. brucei rhodesiense* by inhibiting the ATPase activity. Nevertheless, further studies are needed to better assess the influence of artemisinin on membrane pumps in relation with calcium for these parasites[39]. The University of Cumana in Venezuela performed in 2013 an evaluation of the impact of *A. annua* on epimastigotes of *T. cruzi*. After a treatment of 7 days the density of parasites significantly decreased. Two types of infusions were used: one with leaves from plants grown in Venezuela, the other with leaves from Luxembourg. A dose dependant effect on proliferation was noticed. The infusion from Luxembourg had a stronger effect, although seeds for the two locations were of the same origin[40].

Immunity and HIV

Several trials run by IFBV-BELHERB and its partners in DRCongo, Uganda and India have given preliminary results indicating that *A annua* raises the CD4+ level. These trials will be completed and published. Low CD4+ values are indicative of a depressed immune system and often concommitant with HIV. Several informal claims in Africa exist that the *A. annua* tea infusions are also able to inhibit HIV. The University of Leiden run *in vitro*

trials with *A. annua* and *A. afra* on validated cellular systems for anti-HIV activity[41]. Infusion of *A. annua* tea were highly active (IC_{50} 0.2 µg/mL) while artemisinin was inactive at 25 µg/mL. In addition, it was found that *A. afra* which does not contain artemisinin, showed the same effect as *A. annua.*

Cancer

Ten years ago several research teams claimed that artemisinin was an excellent cure against different forms of cancer. Nevertheless, their claims were based exclusively on *in vitro* trials. No *in vivo* trial with artemisinin or its derivatives has ever been run against cancer. More disturbing is the fact that several *in vitro* trials indicated that some cancer cells developed an invincible resistance against artemisinin, and that would mean that *in vivo* patients might be irremediably lost[42]. There are however many anecdotical reports which show that *A. annua* consumed as infusión or powder cured or stabilized the cancer of a sizeable number of persons. Other molecules different to artemisinin may contribute to the cytotoxic effect against tumor cells. This of course needs to be confirmed by clinical *in vivo* trials.

Dengue and other viruses

A. annua possess a wide variety of anti-viral effects mainly *Herpes simplex* virus Coronavirus and dengue virus. In 2014, a female patient with dengue fever in Vanuatu and her relatives were cured after consumption of *A.annua* infusion (origin of the herb: Luxembourg) (P. Lutgen personal communication). Some *in vitro* data had already been published[43]. These findings need of course to be confirmed by clinical trials in accordance with the WHO protocol.

Antimicrobial

Different extracts and metabolites isolated from *A. annua* have been tested *in vitro* against several Gram positive and Gram negative bacterias and fungi (Table 1), demostrating activity in some microorganisms mainly *Bacillus subtilis*, *Salmonella enteritides* and *Candida albicans*.

Diabetes

A. herba-alba is widely used in Iraqi folk medicine for the treatment of diabetes mellitus most probable because *A. herba-alba* is able to reduce blood sugar. Oral administration of 0.39 g/kg body weight of the aqueous extract of the leaves or barks produced a significant reduction in blood glucose level[44].

Cholesterol

Artemisia species are able to decrease lipid levels similar to that obtained with statins. Thus, for example, aqueous extracts of *A. sieberi* significantly decrease levels of cholesterol, HDL, LDL and triglycerides in diabetic rats[45]. Similar effects have been observed by researchers at the Université des Montagnes in Cameroon with *A. annua* (R Chougouo, personal communication).

Currently, only artemisin and its derivatives are being tested in humans not only for malaria but also for other infectious diseases (Table 2).

Other traditional veterinary, agricultural or insecticidal uses of *A. annua* L.

As the purpose of this review is to cover mainly the applications of *A. annua* in human health we will just mention the best known of other uses. Although the plant contains many nutrients, mammals often avoid it because of its bitterness which will boost their health and immune system. One of the best uses is the fight against

coccidiosis in poultry and other nematodes in cattle and goats[46]. *A. annua* has a bactericidal effect in contaminated water[47] and reduces the frequency and severeness of gastrointestinal diseases.

Artemisia plants are known as repellents for insects in agriculture or in-house when used in fumigation. They also have insecticidal properties[48-50]. Essential oils of *A. annua* have fungicidal properties[51]. Unfortunately the *Artemisia* plants also have allelopathic properties which may negativily impact on other plants growing in their vicinity.

CONCLUSIONS

Plant derived products have been at the origin of many pharmaceutical drugs still in use. Their potential to offer new molecules and drugs is fully recognized today. In their study Newman and Craig from the National Cancer Institute in the US covering 1330 molecules discovered and authorized in the time span 1981-2010, 64% of these were connected in a way or other to plants. For the drugs used for their antibacterial, fongicidal, antiparasitic and antiviral properties this percentage is 68.4%. For anti-diabetical drugs the percentage is 79%[52]. The importance of phytotherapy in the pharmaceutical field is steadily increasing. It is related to the belief of many people that natural products are safer.

A. annua has been used for more than 2000 years. Noboby ever noticed a toxic effect for this plant. This applies also for *A. annua* used as tea in infusions or as powder in capsules. Most of the toxicity studies even have noticed a beneficial effect on many essential functions of the body like liver, heart or kidney. The interest is growing to study the pharmacological properties of other species of the Artemisia genus. IFBV-BELHERB has plantations of *A. vulgaris, A. afra, A. absinthium, A. abrotanum, A. herba alba, A. marítima, A. apiacea,* and *A. campestris* in Luxembourg but also in other countries in Africa. Some of them contain strong antimalarial molecules like thujone, luteolin, nerolidol. Too much time was probably spent on artemisinin and its derivatives, neglecting these other molecules.

Several research groups introduced the concept of Reverse Pharmacology in the search for new molecules or drugs. For plants which have been used for generations in therapy without any side-effect preclinical studies on toxicity appear to be superfluous. But more care is recommended for plants which have no known traditional use. This is in conformity with the WHO strategy for medicinal plants published in December 2013. WHO in this document recognizes that nowadays more than 100 millions of Europeans rely on traditional or complementary medicine (T&CM). For African countries it is estimated that 80% of the people only have access herbal medicine. In the United States US$ 83.1 billions were spent on herbal drugs in 2013 and the market is growing at the rate of 20%[53]. In Colombia it is difficult to assess the size of the market for phytomedicine. The means used by the INVIMA for the survey in this field are insufficient. The market operations are a ballpark of about 600 000 million pesos and has grown at a rate of 50% in the last 3 years as reported by Asociacion Nacional de Naturistas (ASONATURA). A study performed by the Universidad Nacional de Colombia revealed that most of the plants used are imported and strongly recommends that more resources be devoted to local medicinal plants[54]. It is important to protect the biodiversity in our country in order not to loose products which fail to have been discovered. Each plant which disappears is an irremediable loss for humanity and its sufferings.

Conflict of interest

The author declares no conflict of interest.

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Table 1. *In vitro* pharmacological evaluations for *A. annua* and derivatives

Microorganism	Essential oil		Methanol extract		Artemisinin derivatives	
	MIC (mg/mL)	References	MIC (mg/mL)	References	MIC (mg/mL)	References
Gram-positive						
Staphylococcus aureus	32	58	0.25	59	0.09	55
Enterococcus faecalis	26	57	-	-	-	-
Micrococcus luteus	-	-	0.5	59	-	-
Bacillus cereus	53	574	0.5	59	-	-
Bacillus subtilis	0.00781	12	0.5	59	0.09	55
Bacillus thuringensis	0.0313	12	-	-	-	-
Bacillus Pumilus	-	-	0.5	59	-	-
Bacillus sp	26	57	-	-	-	-
Sarcina lutea	2.5	12	-	-	-	-
Gram-negative						
Escherichia coli	64	58	-	-	-	-
Shigella sp.	20	12	-	-	-	-
Salmonella enteritides	5	12	-	-	-	-
Klebsiella pneumoniae	20	12	-	-	-	-
Pseudomona aeruginosa	25	12	2.0	59	-	-
Salmonella sp	-	-	-	-	0.09	55
Helicobacter pylori	-	-	-	-	0.00025-0.001	56
Fungal stains						
Candida albicans	2	58	-	-	-	-
Saccharomyces cerevisiae	2	58	-	-	-	-
Aspergillus fumigatus	5	12	-	-	-	-

Table 2. Interventional studies of *Artemisia annua* derivatives in clinical trials*

Condition	Intervention	NCT identifier	Locations
Schizophrenia	Artemisinin Placebo	01391403	China
Schizophrenia, Schizoaffective disorder	Artemisinin Placebo	00753506	Maryland (US)
Malaria, vivax	Tafenoquine Dihydroartemisinin+Piperaquine tetraphosphate Artemether + Lumefantrine	02184637	Maryland (US)
Malaria HIV Infection	Dolutegravir Artemether - Lumefantrine combination Artesunate - Amodiaquine	02242799	Uganda
Malaria, vivax	Mefloquine - Artesunate Artemether - Lumefantrine Chloroquine	01107145	Brazil
Malaria, falciparum Malaria, vivax	Amodiaquine plus Artesunate; Artekin	00157885	Indonesia
HIV Infection Malaria	Artemether / Lumefantrine	01728961	Malawi/ Uganda
Malaria, vivax	Chloroquine Dihydroartemisinin / Piperaquine	01887821	Vietnam
Malaria, falciparum HIV infections	Artemether - Lumefantrine	00885287	Tanzania
Malaria, vivax	Dihydroartemisinin / piperaquine + primaquine Artesunate – amodiaquine + primaquine	01288820	Indonesia
Cytomegalovirus Infections	Artesunate	00284687	Israel
Malaria, vivax	Artemether-Lumefantrine combination Primaquine Chloroquine	01680406	Ethiopia
HIV Infections	Etravirine Darunavir / Ritonavir Artemether / Lumefantrine	01876966	No locations Provided
Hepatocellular Carcinoma	Artesunate	02304289	Belgium
HIV Infections Malaria	Artemether - Lumenfantrine	00869700	South Africa
Malaria HIV Infections	Dihydroartemisinin - Piperaquine Artemether - Lumefantrine Trimethoprim - sulfamethoxazde	00527800	Uganda

*Adapted from www.trials.gov

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Figure 1. *Artemisia annua* L. plantation (IFBV-BELHERB)

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Figure 2. Encapsulated powdered leaves of *Artemisia annua* L. (IFBV-BELHERB)