

## RESEARCH AND MEDICINAL POTENTIAL OF ARTEMISIA ANNUA: A REVIEW

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### Summary

*Artemisia annua* also known as sweet Annie or Wormwood belonging to the family Asteraceae and the genus *Artemisia*, widely distributed in Asia, Europe and North America, used in Chinese traditional medicine for over 2000 years, is an annual herb. The main chemical constituent obtained from *Artemisia annua* is a hydrophobic sesquiterpene lactone called Artemisinin which is responsible for the anti malarial action. Artemisinin are most important class of anti – malarials because they are potent rapid – acting blood Schizotocides and are effective against multi – drug resistant parasites and hence can be used to treat severe malaria. In addition to their anti – malarial activity artemisinin and its derivatives are also active against Cancer cells. *Colletotrichum species*, found in *Artemisia annua* produces metabolites with activity against human – pathogenic fungi and bacteria as well as the metabolites fungistatic to plant – pathogenic fungi. Other constituents like Arteether, Artemether, Artemisininic acid, Artemisunate, Qinghaosu I–V are also present.

**Keywords:** *Artemisia annua*, Anti – malarial, Phytochemistry, Pharmacology, Review.

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### Introduction

The future of medicine is rooted in the past, before chemists undertook to synthesize synthetic silver bullets for all that ails, and before pharmaceutical companies hitched our collective health to what has become for them a multibillion-dollar wagon<sup>[1]</sup>. In the past, almost all the medicines were from the plants, the plant being man's only chemist for ages. Herbs are staging a comeback, herbal 'renaissance' is happening all over the globe and more and more people are taking note of herbal therapies to treat various kinds of ailments in place of mainstream medicine. There are three main reasons for the popularity of the herbal medicines. 1) There is growing concern over the reliance and safety of drugs and surgery, 2) Modern medicine is failing to effectively treat many of the most common health conditions, 3) Many natural measures are being shown to produce better results than drugs or surgery without the side effects<sup>[2]</sup>.

Malaria kills a child every few seconds: most of the people who die from are children not yet 5 years of age. Thus, control of this disease has now attained utmost attention. And if such a disease has an herbal solution, then it is surely a boon to mankind. Drug – resistant malaria is a major worldwide public health problem. In Southeast Asia, *Plasmodium falciparum* strains have become resistant to all of the classical Antimalarials<sup>[3]</sup>. Fortunately, these strains are still susceptible to the artemisinin derivatives; derivatives such as artemether and artesunate are now widely used in this region<sup>[4]</sup>. Artemisinin it is a naturally occurring endoperoxide with antimalarial properties<sup>[5]</sup>, so has been used clinically as an anti-malaria drug<sup>[6]</sup>. Artemisinin or Qinghaosu is an extract of medicinal plant Qin hao (*Artemisia annua*) (refer picture)<sup>[7]</sup>, a herb which has been used in traditional Chinese medicine for over 2000 years<sup>[8,9]</sup>. Artemisinin rapidly killing all asexual stages of *Plasmodium falciparum*<sup>[10]</sup>. *Artemisia annua* is an annual herb which is widely distributed in Asia, Europe and North America<sup>[11]</sup>.

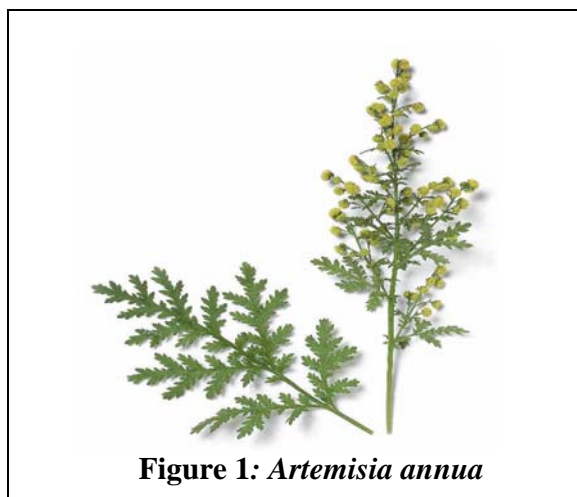


Figure 1: *Artemisia annua*

### Vernacular names

Sweet Wormwood, Sweet Annie, Sweet Sagewort, Annual Wormwood<sup>[12]</sup>. Qing Hao<sup>[7, 13, 14]</sup>, Tung-Pao, Worm weed<sup>[15]</sup>, Huang hua hao, Chenoi<sup>[16]</sup>.

### Taxonomy <sup>[12, 17]</sup>

The plant *Arterisia annua* belongs to the

<b>Kingdom</b>	: <i>Plantae</i> -Plants
<b>Sub-Kingdom</b>	: <i>Tracheobionata</i> - vascular plants
<b>Superdivision</b>	: <i>Spermatophyta</i> - seed plants
<b>Division</b>	: <i>Magnoliophyta</i> - flowering plants
<b>Class</b>	: <i>Magnoliopsida</i> - Dicotyledons
<b>Sub-class</b>	: <i>Asteridae</i>
<b>Order</b>	: <i>Asterales</i>
<b>Family</b>	: <i>Asteraceae</i> - Aster family
<b>Genus</b>	: <i>Artemisia L.</i> - sage brush
<b>Species</b>	: <i>Artemisia annua L.</i> - sweet sage wort
<b>Binomial nomenclature</b>	: <i>Arterisia annua</i>

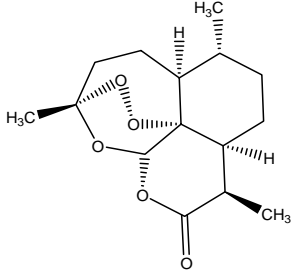
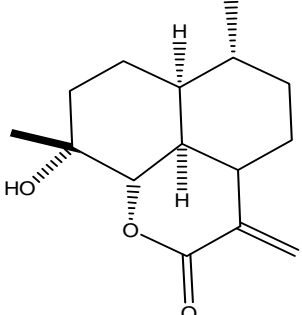
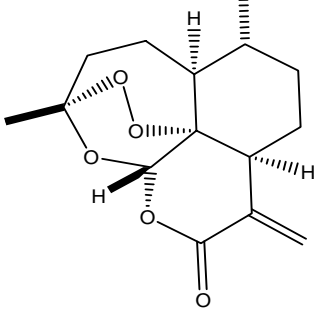
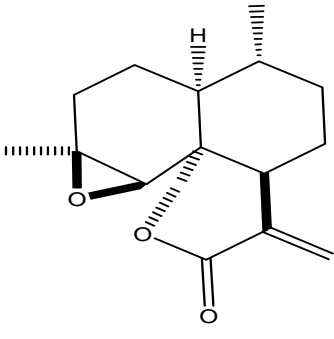
### Morphology <sup>[12]</sup>

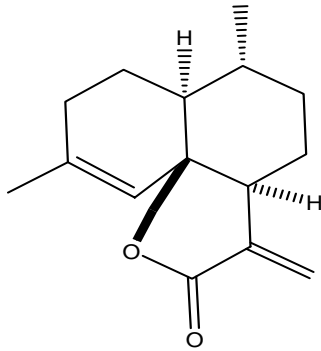
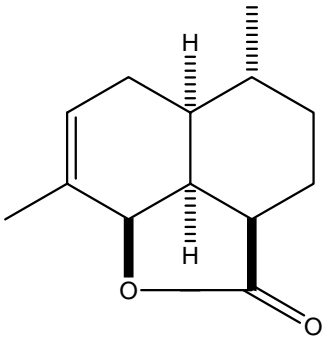
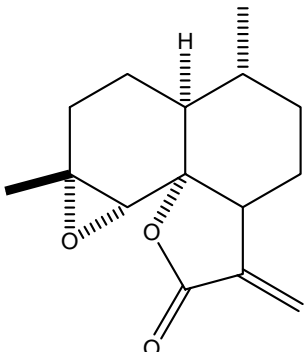
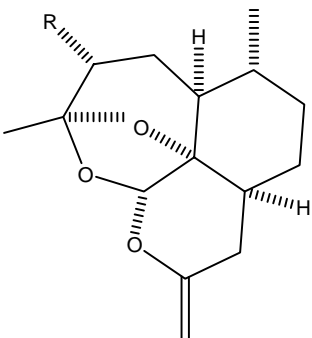
*Artemisia annua*, an annual herb has fern-like leaves, bright yellow flowers, and a camphor-like scent. Its height averages about 2 m tall. The plant has a single stem, alternating branches, and alternating leaves which range 2.5-5cm in length. It is cross-pollinated by wind or insects. It is a diploid plant with chromosome number,  $2n=36$ . Artemisinin may be extracted using a low boiling point solvent such as diethyl ether and is found in the glandular trichomes of the leaves, stems, and infloresces, and it is concentrated in the upper portions of plant within new growth (Figure 1).

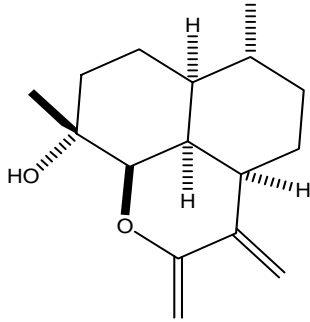
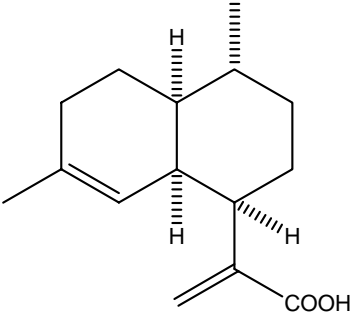
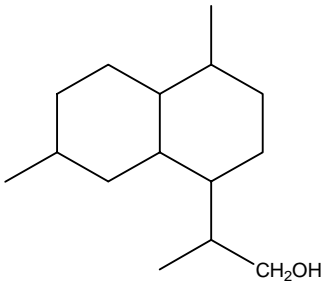
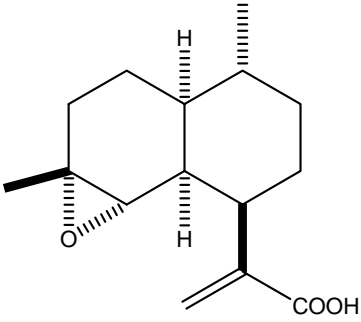
### Phytochemistry

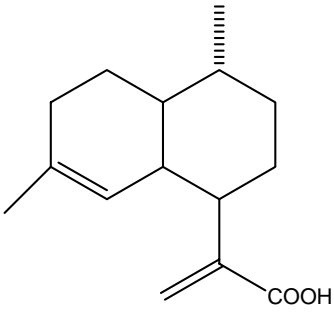
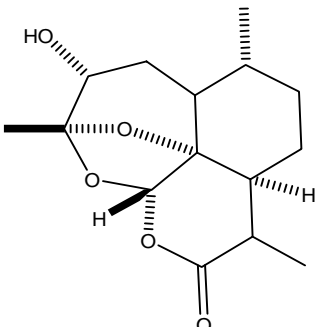
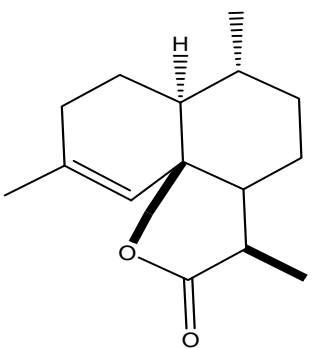
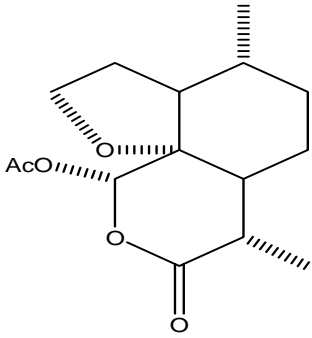
The plant *Artemisia annua* is rich in terpenoids <sup>[18]</sup>. Among these are the sesquiterpene lactone artemisinin, which is one of the most efficient drugs against *Plasmodium* species involved in malaria <sup>[19]</sup>, and monoterpenes camphor, 1, 8-cineole,  $\alpha$ -pinene, and  $\beta$ -pinene, etc. <sup>[20]</sup>, which give the plant a sweet scent. A key enzyme for artemisinin synthesis, amorpha-4, 11- diene synthase, has recently been purified, and its cDNA was cloned <sup>[21]</sup>. At least two monoterpene synthases (QH1 and QH5) were proved to be inducible at the transcriptional level by mechanical wounding. In vitro assay showed that both of them converted GPP into (3R)-linalool, which, however, could not be detected from the plant extract <sup>[22]</sup>. The herb *Artemisia annua* contains artemisinin(a sesquiterpene lactose), arteannuins A and B (qinghaosu I and II), artemisitene, arteannuin C, Epideoxyarteannuin B, artemisilactone, Qinghaosu III, IV and V, Artemisic acid, Artemicinol, Epoxyarteannuinic acid, Artemisininic acid, Arteannuin D, Dihydro – epideoxyarteannuin B, Artemisinin G, Annuadiepoxide, Annulide, Norannuic acid, Artemether, Arteether, ArteannuinB:1, Artemisitene:2,etc (Table:1) <sup>[16]</sup>.

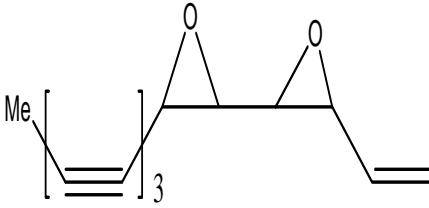
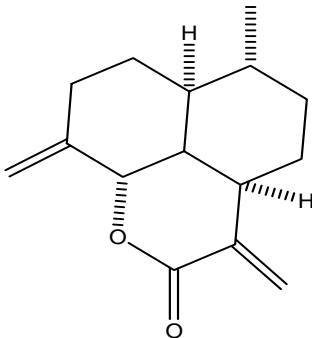
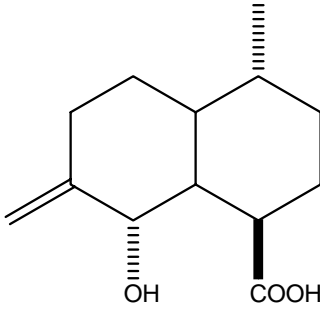
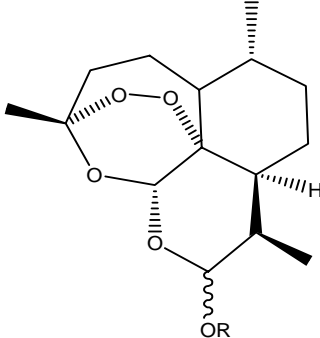
Table 1: Structures of Chemical Constituents Present <sup>[16]</sup>.

Sr.No.	Name Of Compound	Structure
1.	Artemisinin	 <p>The structure of Artemisinin is a sesquiterpene lactone. It features a bicyclic decalin core with a fused six-membered ring containing an oxygen atom. A lactone ring is fused to the decalin system. The structure is highly substituted with several methyl groups and a vinyl group, all shown with specific stereochemistry using wedges and dashes.</p>
2.	Artemisilactone	 <p>The structure of Artemisilactone is a sesquiterpene lactone. It consists of a bicyclic decalin core with a fused six-membered ring containing an oxygen atom. A lactone ring is fused to the decalin system. The structure is substituted with a methyl group, a hydroxyl group, and a vinyl group, with stereochemistry indicated by wedges and dashes.</p>
3.	Artemisitene	 <p>The structure of Artemisitene is a sesquiterpene lactone. It features a bicyclic decalin core with a fused six-membered ring containing an oxygen atom. A lactone ring is fused to the decalin system. The structure is substituted with a methyl group, a hydroxyl group, and a vinyl group, with stereochemistry indicated by wedges and dashes.</p>
4.	Arteammuin C	 <p>The structure of Arteammuin C is a sesquiterpene lactone. It consists of a bicyclic decalin core with a fused six-membered ring containing an oxygen atom. A lactone ring is fused to the decalin system. The structure is substituted with a methyl group and a vinyl group, with stereochemistry indicated by wedges and dashes.</p>

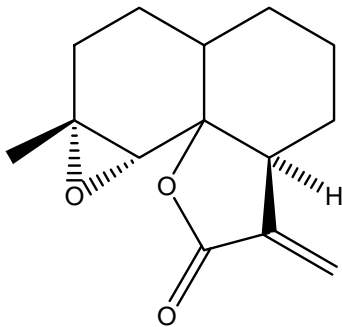
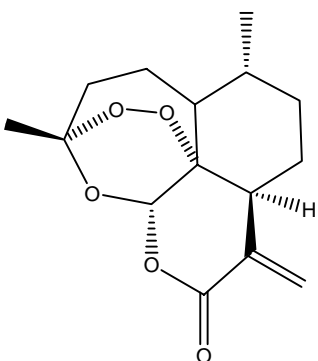
<p>5.</p>	<p><b>Epideoxyarteannuin B</b></p>	
<p>6.</p>	<p><b>Qinghaosu I</b></p>	
<p>7.</p>	<p><b>Qinghaosu II</b></p>	
<p>8.</p>	<p><b>Qinghaosu III (R=H)</b>  <b>Qinghaosu IV (R=OH)</b></p>	

9.	Qinghaosu V	
10.	Artemisic acid	
11.	Artemicinol	
12.	Epoxyasteanuic acid	

13.	Artemisininic acid	 <p>The structure of Artemisininic acid is a bicyclic sesquiterpene. It features a decalin core with a double bond in the left ring and a methyl group at the 2-position. The right ring has a methyl group at the 8-position and a side chain at the 7-position consisting of a methylene group, a methylene group, and a propenoic acid group.</p>
14.	Arteannuin D	 <p>The structure of Arteannuin D is a complex bicyclic sesquiterpene. It has a decalin core with a methyl group at the 2-position and a methyl group at the 8-position. The right ring has a side chain at the 7-position consisting of a methylene group, a methylene group, and a propenoic acid group. The left ring has a hydroxyl group at the 1-position and a methyl group at the 4-position. The structure is highly substituted with various stereocenters.</p>
15.	Dihydro-epideoxyarteannuin B	 <p>The structure of Dihydro-epideoxyarteannuin B is a bicyclic sesquiterpene. It has a decalin core with a double bond in the left ring and a methyl group at the 2-position. The right ring has a methyl group at the 8-position and a side chain at the 7-position consisting of a methylene group, a methylene group, and a propenoic acid group. The structure is highly substituted with various stereocenters.</p>
16.	Artemisinin G	 <p>The structure of Artemisinin G is a bicyclic sesquiterpene. It has a decalin core with a double bond in the left ring and a methyl group at the 2-position. The right ring has a methyl group at the 8-position and a side chain at the 7-position consisting of a methylene group, a methylene group, and a propenoic acid group. The structure is highly substituted with various stereocenters.</p>

17.	Annuladiepoxyde	
18.	Annulide	
19.	Norannuic acid	
20.	<b>Artemether</b> <b>(R=CH<sub>3</sub>);</b> <b>Arteether</b> <b>(R=C<sub>2</sub>H<sub>5</sub>);</b> <b>Artesunate(Na)</b> <b>(R=COCH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Na)</b>	



21.	Arteannuin B:1	
22.	Artemisitene: 2	

### Pharmacological activity

**1. Anti-malarial activity:** Artemisinin is our most important class of antimalarials because they are effective against multidrug-resistant parasites and they can be used to treat severe malaria<sup>[23]</sup>. They kill parasites in asexual stages of infection and prevent the early development of gametocytes, but they also suffer from several limitations. Most artemisinins in current use are semisynthetic derivatives of artemisinin that must be extracted from sweet wormwood (*Artemisia annua*)<sup>[24]</sup>. Artemisinin reveals profound activity against *Plasmodium falciparum* and *Plasmodium vivax*<sup>[25, 26]</sup>. Artesunate and artemether are semi-synthetic derivatives of artemisinin with improved pharmacological features<sup>[27]</sup>. Action of artemisinin is mediated by interaction with parasitic heme, which exerts oxidative stress on the erythrocyte membrane thiols, through the generation of free radicals<sup>[28-30]</sup>. The most active and common metabolite of all artemisinin compounds is dihydroartemisinin, which rearranges to generate a hydroperoxide containing aldehyde and keto groups, which is responsible for parasite destruction<sup>[28,31]</sup>.

**2. Antitumour activity:** In addition to their antimalarial activity, artemisinin and its derivatives are also active against cancer cells<sup>[32-36]</sup>. In both cases, the activity of the drugs is associated with the presence of iron. Iron is present in large excess bound to hemoglobin in erythrocytes, where the Plasmodia parasites are located. The active moiety of artemisinin-like drugs is an endoperoxide bridge, whose reductive homolysis is promoted by iron (II)-heme leading to C4-centered alkylating radicals<sup>[37]</sup>. These radical molecules cause macromolecular damage by alkylating essential malarial proteins inducing cell death of parasites<sup>[38-40]</sup>. On the other hand, iron content is higher in tumor cells than in normal cells<sup>[41]</sup> making them more susceptible to artemisinins. We and others have shown that the susceptibility of tumor cells to artemisinins can further be enhanced by the addition of transferrin or ferrous iron<sup>[42,43]</sup>. The role of artemisinins and iron for malaria

treatment has been intensively investigated during the past years<sup>[44]</sup>, whereas the role of iron for tumor treatment with artemisinins is far less understood. The iron-binding protein, transferrin, is internalized into cancer cells after binding to the transferrin receptor (TfR, CD71). This is a transmembrane glycoprotein involved in iron uptake by internalization of transferrin. TfR exerts growth regulatory functions and is over-expressed in rapidly growing tumors<sup>[45]</sup>. The expression of TfR is of prognostic significance for several tumor types<sup>[46-48]</sup>. Ferrous iron can either be bound to transferrin or to other proteins before uptake<sup>[49]</sup>. Artemisinin and quercetagenin-6, 7, 3', 4'-tetramethyl ether exhibited significant cytotoxicity against P-388, A-549, HT-29, MCF-7 and KB tumor cells<sup>[16]</sup>. Artemisinin also lowered VEGF expression by tumour cells and *KDR* expression by endothelial cells. Artemisinin also has anticancer activity through other pathways. It inhibits the activation of nuclear factor kappa-B (NF- $\kappa$ B), an important activator protein in cancer development and progression<sup>[50]</sup>.

**3. Other activities:** Artemisinin is active against systemic lupus erythematosus and is virustatic against influenza virus in chick embryo. Artemisinin related trioxanes have anti – HIV activity. The plant exhibits anti – oxidative activity and causes cytotoxicity on P388 and L1210 leukemia cell lines. Plant extract exhibited high inhibition of tobacco viruses, showed anti–inflammatory, antibacterial and antinoceptive actions. Artemisic acid was identified as antibacterial and scopoletin as anti–inflammatory agents. Bis-(1-hydroxy-2-methylpropyl) phthalate inhibited germination of wheat coleoptile and mustard seeds. Artemisinin compounds (artemether, artesunate, dihydroartemisinin)<sup>[6, 7]</sup>, all are potent rapid-acting blood schizonticides with elimination half-lives from around 40 minutes to several hours<sup>[51]</sup>. Some flavones, e.g. casticin, chrysoplenetin, chrysosplenol- D and cirsilineol markedly enhanced the antimalarial activity of artemisinin<sup>[52]</sup>. This plant is also known to be Allelopathic<sup>[53, 54, 55]</sup>.

### Phytochemical Analysis

Artemisinin and related compounds have been detected by a number of conventional methods, which include TLC<sup>[56-61]</sup>, HPLC with UV detection<sup>[61-65]</sup> and with electrochemical detection (HPLC-EC)<sup>[66-69]</sup>, gas chromatography (GC)<sup>[70,71]</sup> and GC combined with mass spectrometry<sup>[72-74]</sup>, and MS/MS<sup>[77]</sup>. Unconventional techniques include radioimmunoassay (RIA)<sup>[76]</sup> and enzyme-linked immunosorbent assay (ELISA)<sup>[77]</sup>. The Artemisinin can be estimated by various procedures reported in literature. In TLC–densitometric method the Precoated silica gel plates 60 F<sub>254</sub> of 0.25mm thickness as the Stationary phase and n – hexane – diethyl ether (1:1) is used as the mobile phase. In HPLC method Phosphate buffer – MeOH (6:4) was used as the mobile phase<sup>[28]</sup>.

### Marker Compounds<sup>[28]</sup>

Some of the marker compounds obtained from *Artemisia annua* are

**Artemether:** Its molecular weight is 298.4. It is present in the form of White crystals or White crystalline powder which is almost odourless. Its identification can be done by (a)TLC comparison with standard, (b) IR–spectrum, (c)dissolve 20 mg in 5 ml ethanol, place on a dish and add 1 drop of vanilline–sulphuric acid reagent – a pink colour is produced. Artemether is practically insoluble in water, very soluble in dichloromethane and acetone, freely soluble in ethyl acetate and ethanol. Its melting point is 86–90 °C. The pH of 1% suspension is 3.5 – 5.0. Its sulphated ash content should not be more than 0.1%. Loss on drying should not be more than 0.5%. Heavy metals should not be more than 20 ppm. The related impurities should not be more than 1.0%. It should be stored in air tight containers in cool and dry place.

**Arteether:** Its Molecular weight is 312.4. It is almost white crystalline powder. It is identified by (a)TLC comparison with reference standard. (b)Comparison of IR and UV spectrum with reference standard. It is practically insoluble in water, sparingly soluble in ethanol and methanol, soluble in Arachis oil. Its melting point is 81-84 °C. Loss on drying should not be more than 0.5%. Its sulphated ash content should not be more than 0.1%. The pH of 1% suspension is 3.5–5.0. Other related impurities should not be more than 1.0%. It should be stored in a well closed container in cool and dark place.

**Artesunate:** Its Molecular weight is 384.4. It is a white crystalline powder. It is mainly identified by (a) IR and UV spectrum, (b) TLC comparison (c) Dissolve 0.1 gm artesunate in 40ml anhydrous ethanol, add 0.5 ml hydroxylamine hydrochloride, 0.25ml 8% NaOH, heat to boiling, cool and add 2 drops of dilute HCl and 2 drops of 5.0% FeCl<sub>3</sub> – a red violet colour is produced. Its Melting Point is 132-135 °C. Loss on drying should not be more than 0.5%. It should not contain more than 0.1% sulphated ash. Not more than 0.001% heavy metals should be present. The pH of 1.0% suspension is 3.0–5.0. Other related impurities should not be more than 1.0%. It should be stored in well closed container in dark and cool place.

### DOSAGE <sup>[28]</sup>

#### 1. Artemether

In Tablets : 40mg/50mg  
In Capsule : 40 mg/ 60 mg/ 80 mg/ 100 mg per ml

#### 2. Artemisinin

In Tablets : 250 mg  
In Capsule : 250 mg

#### 3. Arteether

In Injectables : 50 mg/ 75 mg/ 150 mg per ml.

#### 4. Artesunate

In Tablets : 50 mg

### Toxicity Studies

All artemisinin analogues are associated with embryotoxicity over a narrow dose range in the rat and rabbit where embryo-lethality, late resorption and morphological abnormalities (such as abnormal development of the cardiovascular system, axial skeleton and limbs) have been reported <sup>[7, 78]</sup>. There is also some evidence from animal models that artemisinins given later in pregnancy have adverse effects on foetal body weight and survival<sup>[79]</sup>. The mechanism of developmental toxicity in animals is unclear. In rats, the yolk sac is highly susceptible to artemisinin compounds <sup>[79]</sup>, but recent studies in monkeys, which showed high rates of foetal loss, indicate that other mechanisms must be involved in higher mammals <sup>[80]</sup>. In vivo studies in different animal models revealed brain stem neurotoxicity after repeated treatment with high doses of some artemisinin

derivatives over at least 7 days<sup>[81]</sup>. However, repeated treatments with high doses of artemether once every 2 weeks, the recommended dose schedule for the prevention of *S. japonicum* infection, revealed no neurotoxicity<sup>[82]</sup>. Most importantly, there is no clinical evidence of neurological lesions, although several million people have been treated with an artemisinin derivative for malaria<sup>[83]</sup>. Artemisinin compounds are important for treating multidrug-resistant malaria; however, the possible resorption and abnormalities observed in animal reproduction studies may contraindicate artemisinin use during the first trimester. To evaluate whether artemisinin interferes with developmental outcomes at different periods of pregnancy, Wistar rats were treated by gavage with increasing doses of 7, 35 and 70 mg/kg/day from gestational day [GD] 7 to 13 or 14 to 20. Viable embryos and post-implantation losses, and progesterone and testosterone levels, were monitored in the former treatment group and pregnancy and outcomes data, post-implantation losses and male and female developmental endpoints of the offspring were evaluated in the latter treatment group. Results indicate toxicity for both periods of treatment, with lower sensitivity at later stages of pregnancy. The results showed that dosing with 35 or 75 mg/kg of artemisinin caused high percentages of post-implantation losses that correlated with a trend to lower maternal progesterone and a significant maternal testosterone decrease. These findings demonstrate that oral administration of artemisinin can adversely affect post-implantation development and pregnancy in the rat<sup>[84]</sup>. Skin contact with the plant can cause dermatitis or other allergic reactions in some people. Likely to be toxic as are other members of the genus.<sup>[85]</sup>

### Drug Interactions

#### 1. Interaction of Artemisinin with Phenytoin:<sup>[86]</sup>

The aim of the study was to determine the effect of artemisinin, artemether, and arteether on the pharmacokinetics of phenytoin in rabbits. Results suggest that artemisinin compounds alter the pharmacokinetics of phenytoin. Confirmation of these results in human studies will warrant changes in phenytoin dose or frequency, when either of these antimalarials is coadministered with it.

#### 2. Interactions between artesunate and artemisinin:<sup>[87]</sup>

The study carried out on healthy Vietnamese males showed that Artesunate (dihydroartemisinin) did not alter the elimination of artemisinin. However, dihydroartemisinin elimination was inhibited by artemisinin. Artemisinin induced its own elimination even 5 days after a single oral dose. There was no evidence for the formation of dihydroartemisinin from artemisinin.

#### 3. Interaction with Triclosan or Ketoconazole combinations:<sup>[88]</sup>

Interactions of artemisinin with triclosan or ketoconazole against blood stages of *P. falciparum* by a fixed-ratio isobologram method were evaluated. Artemisinin shows mild synergistic interaction with triclosan and slight to marked antagonism with ketoconazole in vitro. These antiplasmodial interactions, however, require confirmation using in vivo model systems.

### Acknowledgement

We are thankful to the Principal of A.I.S.S.M.S College Of Pharmacy, Kennedy Road , Pune. for providing valuable support.

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