Tariq Aftab · Jorge F.S. Ferreira
M. Masroor A. Khan · M. Naeem *Editors*

Artemisia annua - Pharmacology and Biotechnology

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Chapter 1
How Techniques of Herbal Drug Preparation Affect the Therapeutic Outcome: Reflections on Qinghao 青蒿 (Herba *Artemisiae annuae*) in the History of the Chinese Materia Medica

Elisabeth Hsu

Abstract This chapter summarises an earlier study that detailed in chronological order the translation of all the entries on *qinghao* 青蒿 (and its synonyms *caohao* 草蒿, *chouhao* 臭蒿, *huanghuahao* 黃花蒿, etc.) that Frederic Obringer and I could locate in the premodern Chinese *materia medica* (*bencao* 本草) in the time period between 168 BCE and 1596. The aim of that study (Hsu in Plants, health and healing: Berghahn, Oxford, pp 83–130, 2010) was threefold: it aimed to make a contribution to ethnobotany, the history of Chinese medicine and herbal medical practice. It underlined, first and foremost, that 'herbal' medications are not to be conceived of as 'natural' 'herbs' but as cultural artefacts: the entries on *qinghao* in the Chinese *materia medica* contained detailed information on the culture-specific transformation of plant parts into the drugs that the patient would then consume. This underlined that the so-called 'herbal' medical practice depends not only on plant classifications that are culture specific, but also on practical interventions that treat the plant as a thing. Accordingly, the study of *qinghao* involved not merely attending to the cultural acquisition of knowledges (epistemologies) but also to the techniques and practices of intervening with perceived realities (ontologies). Second, the study highlighted that the practical recommendations of how to use the plant and its various parts changed over time; it remains, to date, one of the first longitudinal studies on a specific item of the *materia medica* in the history of Chinese medicine. Finally, it evaluated the identification of *qinghao* and other *hao* 蒿 in terms of modern botanical taxonomies (as given in the *Zhongyao dacidian* 1986).

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1.1 Qinghao in the *materia medica* literature

This chapter begins with a table that lists the most important contents of all the entries on *qinghao* in the *materia medica (bencao)* literature dating from 168 BCE to CE 1596 that have been translated into English (see Table 1.1). This table evaluates the importance attributed to the disorders that *qinghao* was thought to treat by giving the first-named disorder in a *bencao* entry on *qinghao* a number 1, the second-named disorder a number 2, the third-named disorder a number 3 and so on. In a similar manner, it weights the mode of preparation that the text recommends: the first mode of preparation mentioned in a *bencao* entry on *qinghao* is numbered 1, the second 2 and so on. The simple method of tabling the paradigmatic reading of all the *bencao* texts translated in Hsu (2010), and of weighting the findings, by considering the first-mentioned disorders and preparation methods the most important ones, has yielded some interesting additional results:

1. The *bencao* literature underlined the use of *qinghao* for wound healing during the first millennium. It is the sole application recommended in 168 BCE and remains the first-mentioned one up to the seventh century.
2. The *bencao* literature started to mention *qinghao* as prime drug against fevers and, in particular, intermittent fevers only from the Song dynasty (960–1279) onwards; Su Song 蘇頌 was the first to do this in the *Bencao tujing* (Illustrated Canon) 本草圖經 of 1062 [as cited in the *Zhenglei* (Corrected and Arranged into Categories) *bencao* 證類本草].
3. The *bencao* literature consistently listed *qinghao*'s longevity-enhancing properties. These health-enhancing properties were emphasised particularly in dietetics [e.g. the *Shiliao* (Dietary Therapy) *bencao* 食療本草 of 721–739 by Meng Shen 孟詵], although they existed alongside the other recommendations from the earliest documented times onwards. Recommended as a fresh fragrant food supplement, they were mentioned already in the first extant *bencao,* namely Tao Hongjing's 陶弘景 *Bencao jing jizhu* (Notes to the Canon) 本草經集注 of CE 500.

These diverse usages of *qinghao* are certainly linked to the chemistry of the plant *Artemisia annua* L. and related species, but not merely. Socio-historical developments will have played a role too. One wonders, for instance, to what extent *qinghao's* usage for wound healing, and other herbal medications, as those recorded in Salazar (1999) and Harper (1998: 221–230), arise from an interdependent development of technologies of warfare and medicine in general. Furthermore, it would be interesting to explore how closely *qinghao*'s increased usage for treating intermittent fevers is linked to these intermittent fevers' soaring increase in the Song dynasty (Miyasita 1979; Obringer 2001) due to the newly developed agricultural technologies of wet flooded rice cultivation (Bray 1984: 597–615), which created ideal breeding grounds for the malaria mosquito vector.

Table 1.1 Recommended applications and usages of *qinghao* in the chronologically ordered *materia medica* (*bencao* 本草) literature until 1596

Title of the *materia medica (bencao)*	'Bone breaker', fevers	Wound healing	Enhances longevity	Daemonic *qi* convulsions	Soak in urine	Use it raw	Wring out juice	Food supplement	Description of the living kind	Name
(Mawangdui 馬王堆 'Wushier bing fang' 五十二病方, anon., of 168 BCE; manuscript text that is not actually a *bencao*)		1			x					*qinghao* 青蒿, *qin* 菦
Shennong bencao jing 神農本草 (Shennong's canon of the *materia medica*), anon., of first century CE, (L = lost and no longer extant)	2	1	3							*caohao* 草蒿, *qinghao* 青蒿, *fangkui* 方溃
Mingyi bielu 名醫別錄 (Informal Records of Famous Physicians), anon., of third century, (L)	2	1	3						(x)	*caohao* 草蒿, *qinghao* 青蒿, *fangkui* 方溃
Bencao jing jizhu 本草經集注 (Notes to Shennong's Canon of the *materia medica*) of 500 by Tao Hongjing 陶弘景 (fragments extant)	2	1	3					Fresh, mixed with vegetables	(x)	*caohao* 草蒿, *qinghao* 青蒿, *fangkui*方溃
Xinxiu bencao 新修本草(Newly Revised *materia medica*) of 657–659 by Su Jing蘇敬 (fragments extant)	2	1	3					Ditto	(x)	*caohao* 草蒿, *qinghao* 青蒿, *fangkui* 方溃
Shiliao bencao 食療本草 (*materia medica* for Successful Dietary Therapy) of 721–739 by Meng Shen 孟詵 (fragments extant)	3	2	1 and 5	4	x			Pickles in vinegar		*qinghao* 青蒿
Bencao shiyi 本草拾遺 (*materia medica:* Supplements) of eighth century by Chen Zangqi 陳藏器 (L)	(2?)	3		1	x	(x)	x			*hao* 蒿
Shu bencao 蜀本草 (*materia medica* from the Kingdom of Shu) of tenth century by Han Baosheng 韓保昇 (L)									x	*qinghao* 青蒿, *xin hao* 狃蒿

(continued)

Table 1.1 (continued)

Title of the *materia medica (bencao)*	'Bone breaker', fevers	Wound healing	Enhances longevity	Daemonic *qi* convulsions	Soak in urine	Use it raw	Wring out juice	Food supplement	Description of the living kind	Name
Rihuazi bencao 日華子本草 (*materia medica* of Master Sun Rays) of tenth century by Da Ming 大明 (L)	2	3	1	4	(b) x	(a) x			x	(a) *qinghao* 青蒿 leaves and stalks, (b) seeds, (c) *chouhao* 臭蒿seeds
Bencao tujing 本草圖經 (Illustrated Canon of *materia medica*), of 1062 by Su Song 蘇頌 (L)	1	2		3	x			Mix with vegetables	x	*caohao* 草蒿, *qinghao* 青蒿, *fangkui* 方溃
Zhenglei bencao 証類本草 (*materia medica* Corrected and Arranged into Categories) of ca 1082 by Tang Shenwei 唐慎微	2	1	3	4	x	x	x	Mix with vegetables, pickles in vinegar	x	*caohao* 草蒿, *qinghao* 青蒿, *fangkui* 方溃, *xinhao* 狃蒿*chouhao* 臭蒿
Bencao yanyi 本草衍義 (Dilations upon the *materia medica*) of 1116 by Kou Zongshi 寇宗奭								Use as a vegetable	x	*caohao* 草蒿, *qinghao* 青蒿, *xianghao* 香蒿, *changhao* 常蒿
Zhenzhu nang buyi yaoxing fu 珍珠囊補遺藥性賦 (Pearl Bag with Rhapsodies on the Properties of Drugs) of twelfth century by Li Gao 李杲	1	2			x					*caohao* 草蒿, *qinghao* 青蒿, also *xuanfuhua* 旋覆花, *jinfei* 金沸
Bencao pinhui jingyao 本草品匯精要 (Essentials of the *materia medica*, Classified by Grades) of 1505 by Liu Wentai 劉文泰	1	2	3	4	x	x			x	*caohao* 草蒿, *qinghao* 青蒿, *fangkui* 方溃, *xinhao* 狃蒿, *qin* 鼓, *haoqin* 蒿鼓
Bencao mengquan 本草蒙筌 (Enlightenment of the *materia medica*) of 1565 by Chen Jiamo 陳嘉謨	1	2	4	3	x	x	x		(x)	*caohao* 草蒿, *qinghao* 青蒿
Bencao gangmu 本草綱目 (Classified *materia medica*) of 1596 by Li Shizhen 李時珍	2	1	3	4	x	x	x	Mix with vegetables, pickles in vinegar	x	*qinghao* 青蒿, *caohao* 草蒿, *fangkui* 方溃, *qin* 鼓, *xinhao* 狃蒿, *xiang hao* 香蒿

All these entries in the *materia medica* literature are translated in their entirety in Hsu 2010; for *qinghao* formulas in the formula literature, see Wu, de Vries and Hsu forthcoming

1.2 What is in a Name?

In Chinese, the name for a medication (*yao* 葯) can refer to the raw materials, the living plant and the many stages of preparation that lead to the drug that is finally administered to patients. Several preparation methods were repeatedly mentioned in the *bencao* entries on *caohao* or *qinghao*. Among the most frequently mentioned ones belonged urine, which tended to be used as the watery medium within which the fresh plant materials were to be soaked. Furthermore, the recommendation to administer the drug in its fresh/raw state was sometimes very explicit. Finally, it needs to be borne in mind that in antiquity, the Chinese term *caohao* or *qinghao* did not necessarily refer to the plant as a living kind, although the texts spoke of seeds and leaves, roots and stems. It was only during the tenth century that the entry on *caohao* in a *bencao* text would refer to the plant's different stages of maturation and growth, as did Han Baosheng's 韓保昇 *Shu* (Kingdom of Shu) *bencao* 蜀本草 (cited in the *Zhenglei bencao*). From then on, the terms *caohao* and *qinghao* (and synonyms) were consistently used to refer not only to the cultural artefact that is a medicinal drug, but also to the living kind, namely the plant that grows out in the fields.

1.3 How Techniques of Drug Application Interrelate with the Drug's Therapeutic Efficacy

The techniques that turn natural 'herbs' into culture-specific 'drugs' determine and are simultaneously determined by the drugs' effects. At first sight, the shift that happened in the Song dynasty from *qinghao* as primarily a wound-healing drug to one for treating 'intermittent fevers' (瘧，寒熱) not all intermittent fevers are malarial, and malaria can present otherwise than as intermittent fever) would not appear to be related to technological changes in its application. However, if we take into account the observation that 'daemonic *qi*' can refer to the phenomenon of convulsions, and convulsions are sometimes caused by *Plasmodium falciparum* malarial fever bouts (Hsu 2009) techniques of drug preparation did matter.

The use of *qinghao* for treating 'daemonic *qi*' (*guiqi* 鬼氣) is mentioned for the first time in the same text that recommends soaking the presumably fresh plant materials in urine overnight in order to treat the feverish 'bone steaming' (see the *Shiliao bencao* in Table 1.1). Interestingly, 'daemonic *qi*' becomes soon thereafter the first-mentioned disorder in Chen Zangqi's 陳藏器 *Bencao shiyi* (Dilations) 本草拾遺 (cited in the *Zhenglei bencao*). Its application involves accordingly the ingestion of fresh plant materials: 'In autumn and winter use the seeds, in spring and summer use the sprouts, put them together, pound them into a juice and ingest' (updated and improved translation of Hsu 2010: 97).

Later *bencao* texts of the Song and Ming dynasty then also recommend the use of fresh/raw *qinghao*, sometimes after soaking it in urine, sometimes after pounding it into a juice. In those Song and Ming dynasty texts, intermittent fevers tend to be the first-mentioned disorder. Daemonic *qi* is also mentioned in those later texts but relegated to insignificance; it figures often only as number four (see Table 1.1).

So, in this roundabout way, one can indeed reach the conclusion that, contrary to first impressions, the technology of *qinghao*'s preparation was linked to its therapeutic effects and usage: in the history of the *materia medica* the application of *qinghao*, after pounding seeds and (fresh) sprouts into a juice, was recommended for the first time, in order to treat 'daemonic *qi*'. This application method, which involved the ingestion of fresh *qinghao* juice, remained important for its treating of what eventually became the disorder of primary consideration: intermittent fevers (*nüe* 瘧).

1.4 The Problem of Disseminating Specialised Scientific Knowledge

Meanwhile, the famous physician and alchemist Ge Hong 葛洪 had in the fourth century CE already noted that the soaking of the plant in water in order to subsequently wring it out and ingest the (wrung out) juice was effective in treating intermittent fevers (see Hsu 2010; Wright et al. 2010). Ge Hong had made this recommendation in a different genre, namely that of writing recipes and formularies (*fangji* 方劑), but the compilers of the *materia medica* (*bencao*) did not pay attention to it. It was only after 1,200 years that the observation that Ge Hong 葛洪 had made in the context of writing recipes started to be integrated into the *bencao* literature: Ge Hong's recipe is cited for the first time in Li Shizhen's 李時珍 *Bencao gangmu* (Classified Compendium) 本草綱目 1596.

This final observation provides also the motivation for why this chapter has been written for this volume: it is with the explicit aim to make connections between the different scientific genres that specialised research activity inevitably produces. The hope is that the reader may now turn to the literature that provides detailed information on the above problem in a context that provides a more grounded framework for understanding it.

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Chapter 2
Ethnopharmacology of *Artemisia annua* L.: A Review

Alia Sadiq, Muhammad Qasim Hayat and Muhammad Ashraf

Abstract *Artemisia annua* L. has been recognized as important ethanomedicinal herb since two millennia. It has been included in ancient pharmacopoeias of various Asian and European countries. World Health Organization has recommended *A. annua* as antimalarial drug. Its most common ethnobotanical practice involves the use of whole plant decoction for the treatment of malaria, cough, and cold. Diarrhea is also reported to be cured by taking its dry leaves powder. Whole flowering plant is known to be antihelminth, antipyretic, antiseptic, antispasmodic, carminative, stimulant, tonic, and stomachic. The tincture was formally used to treat nervous diseases and crushed plants in liniments. *A. annua* tea infusion has been used for the treatment of malaria in African countries. *A. annua* contains vital compound known as artemisinin that provide structural chemical base for combinatorial treatment therapy for world antimalarial program. Research studies also report that artemisinin is effective for killing human breast cancer cells. Therefore, isolation and characterization of artemisinin has increased the interest in *A. annua* worldwide. Several ethnobotanical uses in Africa claim that the *A. annua* tea is also effective against HIV. Recently, research investigations are more focused to evaluate its antiviral potential against HIV, as it is highly emerging disease throughout the world. Therefore, scientific validation can provide the support to the concept of "ethnopharmacology in overdrive".

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2.1 Introduction

Artemisia annua L is well-known medicinal plant (Bhakuni et al. 2001; Emadi 2013; Tayebe et al. 2012). *A. annua* is the only planta medica that has been recognized to research and developed as the standards of western medicine research by the WHO in China. It is a famous herb, known for its highest efficiency and lowest toxicity in treating ague (Wang et al. 2011). It is an aromatic annual herbaceous plant (Ellman 2010; Huang et al. 2010; Zanjani et al. 2012; Liu et al. 2013; Misra et al. 2013) belongs to genus *Artemisia* (Liu et al. 2013), family *Asteraceae* (Compositae) (Geldre et al. 2000; Mannan et al. 2010; Tayebe et al. 2012; Zanjani et al. 2012) and commonly known as sweet wormwood or Qinghao (Huang et al. 2010; Emadi 2013). It is the only member of genus Artemisia with an annual growth cycle (Willcox et al. 2004). Qing Hao is an ancient Chinese name for *A. annua,* which means "green herb." There are two different theories exist regarding the origin of name. According to first theory word, *Artemisia* is named after the name of Greek Goddess "Artemis" which literally mean "she who heals sickness." It was belief of local people that she heals the diseases and eliminates the evil. Second theory report that it is named after the Queen's name "Artemisia of Caria". She was the queen of Turkey (Ferreira 2004; Willcox et al. 2004). *A. annua* has remained the part of Chinese traditional medicine more than 2,000 years, and currently, it is endemic to China (Olliaro and Trigg 1995; Ferreira 2004; WHO Monographs 2006; Ellman 2010; Mannan et al. 2010). However, *A. annua* has been recognized all over the world since 1970s after the discovery of only natural phytomedicinal source for production (Huang et al. 2010) of the antimalarial lactone artemisinin. Presently, this important phytoconstituent and its derivatives have been widely explored for cure of drug-resistant malaria (Laughlin 2002; Liu et al. 2013; Emadi 2013; Misra et al. 2013). *A. annua* has been established as crop in agriculture after the statement of World Health Organization, as a valuable component of combinatorial therapy for malaria since 2001 (Ferreira 2007).

2.1.1 Origin

This plant is native of Asia and most appropriately originates in China particularly in Suiyuan and Chahar provinces. China has long history of cultivation of *A. annua* and skillful for its unique method of extraction of artemisinin, hence, it has become first country for isolation of artemisinin from plant extracts. In addition, China has also become the largest country on the global market as a supplier of raw material of *A. annua* (WHO Monographs 2006; Ferreira and Janick 2009; Huang et al. 2010; Sharma et al. 2011; Das 2012). There are very few studies that provide the evidence about its origin. Plant remains have been obtained from the Shengjindian cemetery about 2400–2000BP, Turpan, Xinjiang, China. These records provide a information

about its traditional use in ancient times, when People used stalks and inflorescence of *A. annua* to place in the corner of a tomb. Morphological examination of plant remains, ancient DNA extraction and further comparative analysis with modern specimens, provide the insight that these plant remains were belonging to *A. annua*. Further, it gives the rational insight toward its traditional use in the ancient times. This plant is strongly aromatic so local people used it with the purpose to eliminate the odor of the dead. This is the first evidence, based on the archeological studies. Several other ancient Chinese documented records also mention its numerous herbal uses. It is believed that it is not only indigenous to China but also found as native to Korea, Japan, Myanmar, Northern India, Vietnam, and Southern Siberia throughout Eastern Europe. Afterward, it spread to various other countries of North America and tropical areas (Willcox 2009; Liu et al. 2013). It wildly grows in Australia, Turkey, Iran, and Afghanistan. Now, it is commonly cultivated in Vietnam, Romania, Kenya,Tanzania (Bhakuni et al. 2001; Huang et al. 2010; Khosravi et al. 2011), Argentina, Bulgary, French, Hungary, Italy, Spain, United States, and Yugoslavia (Ferreira and Janick 2009; Lestari et al. 2011). Naturally, *A. annua* cover wide range of subtropical and temperate environments including northern hemisphere (mid to high latitudes). There are also very few representatives in the southern hemisphere (Ellman 2010; Das 2012). Cultivation on experimental basis in temperate and subtropical conditions has been started in India (Bhakuni et al. 2001). Breeding technique has been used to develop specific seed varieties for adapting lower latitudes, and it has been achieved successfully in various tropical countries including Congo, India, and Brazil (Willcox 2009).

Scientific names: *Artemisia annua* L.
Vernacular names
Chinese: Caohao, Cao Qinghao, Cao Haozi, Chouhao, Chou Qinghao, Haozi, Jiu Bingcao, Kuhao, San Gengcao, Xianghao, Xiang Qinghao, Xiang Sicao, Xiyehao
English: annual wormwood, sweet wormwood, sweet annie
French: armoise annuelle
Japanese: Kusoninjin
Korean: Chui-ho, Hwang-hwa-ho, Gae-tong-sook
Vietnamese: Thanh cao hoa vàng.

2.1.2 Pharmacognostical Studies

2.1.2.1 Macroscopic Characteristics

A. annua an aromatic annual herb with deeply grooved branches. Variation generally presents in the leaves and aerial parts. The leaves margins are not entire, but the base is asymmetrical. Leaf color varies from light green to dark green and arranged pinnately. Outer and inner surfaces are glabrous. Glandular and non-glandular trichomes are present on the both surfaces. Spongy parenchyma contains 4–6 layers of loosely arranged cells (Das 2012).

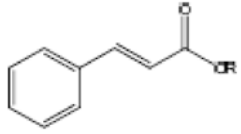
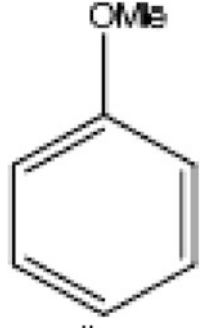
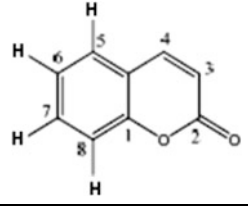
2.1.2.2 Microscopic Characteristics

Physiochemical analysis report average 9.2 w/w moisture content, 8.3 w/w total ash, 0.91 % acid insoluble ash, 6.2 w/w alcohol, and 3.8 v/w water content in *A. annua*. High percentage of protein, crude fat, and digestible fraction is also present in leaves and inflorescence. Plant tissue contains high amount of manganese and copper. Amino acid and vitamin profile are also very high, which increase nutritional value of this herb (Das 2012).

2.1.2.3 Chemical Constituents

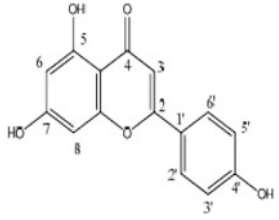
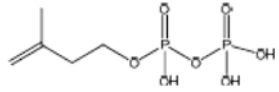
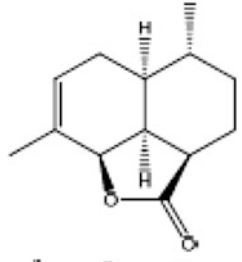
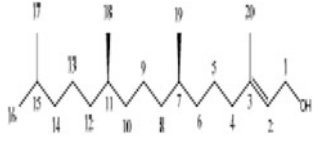
A. annua has become the subject of intensive phytochemical evaluation following the discovery of the antimalarial drug artemisinin (Wang et al. 2011). Phytochemical analysis has identified various compounds including steroids, coumarins, phenolics, flavonoids, purines, triterpenoids, lipids, and aliphatic compounds, monoterpenoids (Emadi 2013; Cafferata et al. 2010; Ferreira et al. 2010), essential oils, alkaloid, and glycoside (Zanjani et al. 2012). Major terpene derivatives such as artemisia ketone (Tellez et al. 1999), artemisinic alcohol, arteannuin B, and myrcene hydroperoxide have been identified. A few of them are also present in essential oil (Verdian-rizi et al. 2008; Brown 2010). Essential oils contain both nonvolatile and volatile constituents. The volatile components of essential oils are camphene, 1-camphor, isoartemisia ketone, β-camphene, β-caryophyllene, β-pinene, artemisia ketone, 1, 8-cineole, camphene hydrate, cuminal (WHO Monographs 2006; Willcox 2009; Das 2012), Artemisia ketone, 1.8-cineole camphor, germacrene D, camphene hydrate, and alpha-pinene, betacaryophyllene, myrcene, and artemisia alcohol (Liao et al. 2006; Ferreira and Janick 2009). The nonvolatile component of essential oil contains sesquiterpenoids (Brown 2010), flavonoids and coumarins, β-galactosidase, β-glucosidase, B-sitosterol, and stigmasterol (Willcox 2009; Cafferata et al. 2010; Das 2012). It also contains erythritol (50.30 %), camphor (7.25 %), pinocarveol (4.13 %), and diethoxyethane (2.18 %) (Haghighian et al. 2008). Scopoletin belongs to the group of coumarins that have been found in *A. annua* extracts (Tzeng et al. 2007). Scopoletin (coumarin), scopolin (comarin glycoside), domesticoside (phloroacetophenone), chrysosplenol-D (flavonoid), and norannuic acid (bisnor-cadinane) are vital phytoconstituents (Emadi 2013; Cafferata et al. 2010). First time, artemisinin (sesquiterpene lacton) isolated from *A. annua* in 1972 (Geldre et al. 2000; Ogwang et al. 2012). Artemisinin is a rare sesquiterpene lactone endoperoxide of the cadinane series (Laughlin 2002). Although there are approximately 400 species of artemisias (Ferreira 2004), artemisinin and essential oil levels in the leaves of *A. annua* ranged from 0.01 to 1.4 % and 0.04 to 1.9 %, respectively (Damtew et al. 2011). The leaves of *A. annua* are only natural source of artemisinin and other vital secondary metabolites (Table 2.1) which can be further used for the production of derivatives of pharmacological importance (Laughlin 2002; Willcox et al. 2004; Brown 2010; Cafferata et al. 2010).

Table 2.1 The phytochemical constituents of *Artemisia annua* L

S. No	Phytochemical groups	Phytoconstituents	Chemical structure	Alternative name (s)
1	Phenylpropanoids	Methyl cinnamate		3-phenyl-2-propenoic acid methyl ester
2	Phenols	Anisole		Phenyl methyl ether
3	Coumarins	2H-1-Benzopyran-2-one		2H-1-Benzopyran-2-one

(continued)

Table 2.1 (continued)

S. No	Phytochemical groups	Phytoconstituents	Chemical structure	Alternative name (s)
4	Flavones	Apigenin		4',5,7-Trihydroxyflavone
5	Monoterpenoids	Isopentenyl pyrophosphate		–
6	Sesquiterpenoids	Artemisinin		Artemisinin I
7	Terpenoids	Phytol		2-Phyten-1-ol (2E, 7R,11R)

Source Brown GD (2010) The biosynthesis of artemisinin (Qinghaosu) and the phytochemistry of *Artemisia annua* L. (Qinghao). Molecules 15: 7603–7698

2.1.3 Ethnopharmacological History

Ethanopharmacological compilation named as "Fifty-two prescriptions" (dates back to 168 BC) mentioned *A. annua* (*qinghao*) as medicinal herb. This document describes it: as a remedy for hemorrhoids that resembles "cow lice" (possibly ticks). Traditional Chinese materia medica (*Shennong ben cao jing*), which was present in the first century AD, but now it has been lost, documented its use as food preservative, remedy for summer heat and for the treatment of "intermittent fevers". This *Handbook of Prescriptions for Emergency Treatment* (Zhouhou Beiji Fang) had been documented in 340 AD, describes number of preparations as traditional medicine (Willcox 2009). Traditionally, it has been used as flavoring agent. Based on this strong traditional use and characteristic fragrance later on, it becomes the potential source for essential oils for the perfume industry (Ferreira and Janick 2009; Huang et al. 2010; Liu et al. 2013). For over 2000 years, the Chinese have used *A. annua* as natural remedy to treat malaria (Geldre et al. 2000; Meier zu Biesen 2010). The *Pharmacopoeia of the People's Republic of China* also describes its use to cure consumptive fever and jaundice (WHO Monographs 2006; Castilho et al. 2008; Ogwang et al. 2012) wound healing and for the improvement of eye brightness (Liu et al. 2013). *A. annua* has also been used traditionally in Iran as medicinal plant for infants as an antispasmodic, carminative, or sedative/ hypnotic remedy (Emadi 2013; Sharma et al. 2011). *A. annua* decoction has been used as antihemorrhage to cure diarrhea (Mirdeilami et al. 2011). Effect of *A. annua* L. on hemostasis is well known in traditional medicine (Wang et al. 2011). Traditional medicinal practices involve usage of different plant parts of *A. annua* to cure different disease (Table 2.2).

2.1.4 Pharmacological Activities

2.1.4.1 Antihypertensive Activity

Antihypertensive potential of aqueous extracts of artemisia leaves of different species, have been assessed by using in vivo models of diabetic rats and rabbits that were administered with dose of 100–390 mg kg^{-1} for 2–4 weeks. Results revealed the significant effects of aqueous extracts by exhibiting the reduction in blood level. Consequently, this action prevents elevation of glycosylated hemoglobin level and produces hypoliposis effect. It also causes the protective effect against body weight loss in diabetic animals. Further, it caused significant inhibition of the phenylephrine-induced contraction, and simultaneously stimulates the endothelium-dependent relaxation of rat aortic rings (Das 2012).

Table 2.2 Medicinal uses of different plant parts of *Artemisia annua* L

S. No	Medicinal uses	Plant part	References
1	Antihemorrhage	Whole plant	Mirdeilami et al. (2011)
2	Diarrhea	Whole plant	
3	Anemia	Stem	Willcox et al. (2004)
4	Damp summer heat with nausea	Root	
5	Intense fever	Rhizome	
6	Stifling sensation in chest	Rhizome/seed powder	
7	Malaria	Leaf/whole Plant	Ogwang et al. (2012)
8	Asthma	Leaf	Anamed international (2011)
9	Eye infections	Leaf	
10	Bronchitis and sore throat	Leaf	
11	Cholera	Leaf	
12	Dengue fever	Leaf	
13	Lupus erythematosus	Whole plant	
14	Athlete's foot and eczema	Leaf	
15	Chagas disease	Leaf	Weathers et al. (2011)
16	Schistosomiasis	Leaf	Zanjani et al. (2012)
17	Viral hepatitis B	Leaf	
18	Chills and fever	Whole plant	Meier zu Biesen (2010)
19	Skin disease	Leaf	Sharma et al. (2011)
20	Parasitic disease including schistosomiasis and leishmaniasis	Leaf	Mannan et al. (2010)

2.1.4.2 Antimicrobial Activity

Research studies have been carried out to evaluate antimicrobial potential of the essential oils obtained from *A. annua*. Experiments revealed that essential oil showed antimicrobial potential against wide range of Gram-negative bacteria, Gram-positive bacteria, and fungi. Significant inhibitory activity of the oil was found against bacterial strains, including *Staphylococcus aureus, Escherichia coli,* and *Enterococcus hirae*. Whereas *Pseudomonas aeruginosa* showed no sensitivity for essential oil. *A. annua* extracts possess remarkable antibiotic potential against fungi particularly *Saccharomyces cerevisiae* and *Candida albicans* (Juteau et al. 2002; Das 2012). Furthermore, these investigations also revealed that essential oils showed more pronounced effects against fungal strains than against Gram bacterial strains (Verdian-rizi et al. 2008). Studies based on chemical evaluation of plant extracts evidenced that phytoconstituents are responsible for conferring this antimicrobial potential. Most vital compounds that have been studied for this bioactive potential are scopoletin (Tzeng et al. 2007), sesquiterpene lactone endoperoxide artemisinin and variety of other derivative compounds. Mechanism of action these Compounds at molecular level have been studied in *Escherichia coli*, *Mycobacterium smegmatis,* and *Mycobacterium tuberculosis*. It has been observed that Arteether acts at nuclear level and hampers the function of DNA-gyrase which is

resistant by quinolone (Kumar et al. 2003). Artemisinic acid is another well-known precursor compound used for semisynthesis of artemisinin, and it has also been studied for antibacterial activity (Bhakuni et al. 2001; Muzemil 2008; Huang et al. 2010).

2.1.4.3 Anti-inflammatory Activity

Anti-inflammatory activity of aqueous methanolic extract has been tested for acute and chronic inflammation by implying variety of inflammatory models. Aqueous extract exhibits anti-inflammatory effect in a dose-dependent manner and resulted in pronounced activity against edema. Phytochemical analysis reports the presence of number of important groups of compounds such as triterpenoids, flavonoid, polyphenols, and coumarin. Therefore, these compounds act additively and impart inhibitory potential against edema response in acute and chronic models (Das 2012). Some other research analysis also report more anti-inflammatory compounds named as scopoletin (a coumarin) (Muzemil 2008), artemisinin, dihydro artemisinin, and arteether. In vivo assays revealed that these compounds significantly inhibit the humeral responses at increased concentration. But some other studies suggest that pure compounds did not show significant efficacy in chronic hypersensitivity response (Bhakuni et al. 2001). Further experimental studies carried out on murine macrophage like RAW 264.7 cell showed the effect of scopoletin in a dose-dependent manner. Therefore, numerous research studies recommend scopoletin as a candidate for anti-inflammatory medicine (Tzeng et al. 2007).

2.1.4.4 Antioxidant Activity

A. annua is a good source of different nutritional constituents and antioxidants (Das 2012). Studies indicate that crude organic extracts of aerial parts have high antioxidant capacity which is most probably due to the fact that leaf contains high content and variety of flavonoids, including the newly reported C-glycosyl flavonoid as a possible component of the antioxidants. Flavonoids and essential oil content present in *A. annua* impart antioxidant potential. Therefore, these studies ranked *A. annua* among those medicinal plants which are at the top of list, based on their highest antioxidant potential (Juteau et al. 2002; Ferreira and Janick 2009). Major groups of hydroxylated and polymethoxylated flavonoids have been identified which further include chrysosplenol-D, cirsilineol, eupatin, chrysoplenetin, cirsilineol, casticin, and artemetin (Ferreira et al. 2010). Studies have identified respective five bioactive flavonoids and further subjected to structural analysis. These include 5-hydroxy-3,7,4′-trimethoxyflavone,5-hydroxy-6,7,3′,4′-tetramethoxyflavonol, blumeatin, 5,4′-dihydroxy-3,7,3′-trimethoxyflavone and quercetin (Yang et al. 2009).

2.1.4.5 Immunosuppressive Activity

A. annua has been evaluated for its immunosuppressive activity. Ethanolic extract of *A. annua* significantly suppressed concanavalin A (Con A) and lipopolysaccharide (LPS)-stimulated splenocyte proliferation in vitro and this activity increases with increase in dose. Results have also showed that ethanol extract of *A. annua* could suppress the cellular and humoral response (Das 2012). Immunosuppressive potential have been linked to flavonoids present in leaves which are capable to modulate the immune response (Ferreira et al. 2010).

2.1.4.6 Antiarthritis Activity

Experimental studies have revealed that artemisinin derivative SM905 (obtained from *A. annua*) suppresses the inflammatory and Th17 responses which cause the improvement in collagen-induced arthritis. These studies have been carried out on collagen-induced arthritis (CIA) by type II bovine collagen model (CII) in DBA/1 mice through oral administration of artemisinin derivative SM905. Incidence of disease and severity were observed regularly. Gene expression and T helper (Th) 17/Th1/Th2 type cytokine production level have also been examined. Observations of this study revealed that SM905 compound play key role as it delayed the onset of disease, hence reduce the incidence of arthritis. Furthermore, it also reduces the overexpression of variety of pro-inflammatory cytokines and chemokines (Das 2012).

2.1.4.7 Antimalarial Activity

Malaria is a global threat since long. In order to deal with this situation, it needs a coordinated approach consist of prevention strategies, therapeutic medicines, and curative treatment of patients. Therefore, extraction of artemisinins from *A. annua* has opened the way toward new and highly effective alternates (Ferreira 2004; Ridder et al. 2008; Ferreira and Janick 2009). *A. annua* L is now well recognized throughout the world (Liu et al. 2009; Willcox 2009), and currently, it is in use over 50 countries as a strong drug substitute against malaria, particularly chloroquine-resistant malaria (Ferreira et al. 2006). Studies have reported many other flavonoids (artemetin, casticin, chrysoplenetin, chrysoplenol-D, cirsilineol, and eupatorin) which possess antiplasmodial efficacy (El-feraly et al. 1989; Lubbe et al. 2012). Mechanism of methoxylated flavonoids is associated with activation of artemisinin, which explains the key role of methoxylated flavonoids, as it facilitates the interaction of artemisinin with plasmodial hemoglobin involving catabolic pathway that produces artemisinin peroxide. Furthermore, artemisinin peroxide inhibits the heme polymerization and ultimately confers the antimalarial effects against protozoan *Plasmodium* species: *falciparum vivax, malariae,* and *ovale*. Another mechanism of flavonoids suggests that it blocks the incorporation of hypoxanthine by *Plasmodium*

(Laughlin 2002; Muzemil 2008; Das 2012). Although artemisinin induce antiplasmodial effects through alkylation of malarial-specific proteins (Bhakuni et al. 2001), some flavonoids had no specific antiplasmodial activities but had capability to potentiate antiplasmodial activity of artemetin (Ferreira et al. 2010). In early 1970s, Chinese scientists have selected artemisinin, artemether, and sodium artesunate for clinical evaluation. There are studies in which malarial patients (more than 3000) were clinically subjected to the treatment by artemisinin and its derivatives. These results suggest more curative potential of artemisinin compounds particularly against drug-resistant *P. falciparum* (Mueller et al. 2000; Weathers and Towler 2012). Comparative clinical studies have been conducted to evaluate the efficacy of whole herb of *A. annua* and chloroquine. Organic extracts *A. annua* have been found more effective, faster, and less toxic than chloroquine in treating malaria (Huang et al. 2010; Tayebe et al. 2012). It significantly reduces parasitemia and improves the immune response by stimulating phagocytic activity of macrophages. Whole plant extract activity is more pronounced because of the presence of various phytoconstituents that impart synergistic antimalarial potential. Therefore, it is quite obvious that current combinatorial approach may be representing the formulations of phytoconstituents (and sometimes plant species) that confer synergistic effect, as they are present in the herbal prescriptions (Willcox 2009; Donno et al. 2012).

2.1.4.8 Antiparasitic Activity

Research studies suggest that artemisinin drugs have good antiparasitic potential for Leishmania, Trypanosoma *Babesia*, *Eimeria* or coccidiosis, trematodal blood fluke *Schistosoma* spp., and *Schistosoma japonicum*, *Schistosoma mansoni,* and *Schistosoma haematobium*. Therefore, currently, its use in livestock industry has been increasing (Kumar et al. 2003; Ferreira and Janick 2009). A study has been conducted against *Neospora canum*, which is a protozoal parasite of mammals. Cultured Vero cells or mouse peritoneal macrophages were infected with of Artemisinin for 14 days. All microscopic foci of *N. caninum* completely eliminated at 20 or 10 μg/ml after 11 days, and same results were obtained at concentration of 0.1 μg/ml. Therefore, artemisinin has potential to reduce the intracellular multiplication of *N. caninum* tachyzoites. In another study, the effect of artemether was tested against the larval stages of *Schistosoma mansoni*. It has been found that animals did not develop schistosomiasis after artemether treatment. Susceptibility of parasite was quite pronounced as compared to the nontreated controls (Das 2012). Recently, another research study reports that n-hexane extracts of *A. annua* leaves and seeds exhibit significant activity against *Leishmania donovani*. This antileishmanial activity includes morphological changes in promastigotes, apoptosis, and cell-cycle arrest at cellular level (Islamuddin et al. 2012).

2.1.4.9 Anticancer Activity

A. annua is well known by its pharmacological applications in the popular medicines, and currently it is a subject of research studies with the aim to find the treatment against cancer (Cafferata et al. 2010). Anticancer activity of various organic extracts of *A. annua* has been evaluated by determining their cytotoxic potential in *Trypanosoma b. brucei* (TC221 cells) and HeLa cancer cells. These evaluations showed that methanol extracts are more cytotoxic as compared to dichloromethane extracts (Efferth et al. 2011). Cytotoxicity studies of artemisinin and quercetagetin-6, 7, 3¢, 4¢-tetramethylether against various tumor cells including P-388, A-549, Ht-29, KB, and MCF-7 cells showed significant efficacy (Bhakuni et al. 2001; Muzemil 2008). In vitro and in vivo anticancer testing exhibits promising results of artemisinins, and further investigations reveal its mechanism of action, which provides an insight toward its constitutional property that is built in its structure. Artemisinin contains an endoperoxide group that imparts anticancer activities. Like some other compounds such as hydrogen peroxide, artemisinin reacts with ferrous iron and make free radical species. These free radicals trigger anticancer activities. Further extended research investigations report that these anticancer activities become more pronounced upon addition of iron complexes in cell culture. Artemisinin makes covalent conjugate with transferrin (an iron transport protein, found in human) so this artemisinin and transferrin conjugate actively transported inside the cancer cells by the involvement of transferrin receptor (TfR)-mediated endocytosis pathway and result in pronounced anticancer activity experimental cell cultures. This also explains the importance of iron metabolism that enhances the anticancer potential of artemisinin. In addition, artemisinin and its derivatives induce programmed cell death in cancer cells through activation of cytochrome C-mediated pathway which lead toward apoptosis (Ferreira et al. 2010). Therefore, several research investigations established artemisinin as s potent anticancer agent (Huang et al. 2010; Nadeem et al. 2013) and recommend it against cancer as drug therapy (Ferreira and Janick, 2009; Ferreira et al. 2010; Zanjani et al. 2012). Chemical and structural characteristics also recommend it as a lead compound, which can further become the basis of drug development (Bhakuni et al. 2001). Research studies have also identified some other vital compounds which possess antitumor activity such as scopoletin (Tzeng et al. 2007), artemisinin and its derivatives (Kumar et al. 2003).

2.1.4.10 Angiotensin Converting Enzyme Inhibitors

Studies have identified few flavonoid compounds from *A. annua* such as fisetin and patuletin-3, 7-dirhamnoside, which exhibit the potential for blocking nonpeptide angiotensin converting enzyme (Bhakuni et al. 2001; Muzemil 2008).

2.1.4.11 Antiviral Activity

Antiviral activity of *A. annua* tea infusions against HIV has been evaluated very first time through scientific investigation. Two independent cellular systems have been used for toxicity studies. The *A. annua* tea infusion exhibits highly significant activity at very low concentration (2.0 μg/mL). But artemisinin was found inactive at higher concentration (25 μg/mL). Similarly, no cellular cytotoxic effects were observed at higher concentration of tea infusion. Therefore, this in vitro study revealed that artemisnin plays limited role and may act synergistically against anti-HIV activity (Lubbe et al. 2012). Some other in vitro studies have claimed about inhibitory effects for hepatitis B virus (WHO Monographs 2006). Currently, artemisinin and its derivatives has become the subject of scientific studies to investigate their potential against number of viruses (Ferreira and Janick 2009) with the aim of advanced combination therapies of antivirals (Weathers and Towler 2012).

2.1.4.12 Plant Growth Regulatory Activity

Research studies report that *A. annua* contain series of vital compounds that have the potential to regulate the plant growth activities and some of them act as natural pesticides. These compounds have also been recommended as natural pesticide in agriculture. These compounds are bis (1-hydroxy-2-methylpropyl) phthalate, abscisic acid, and abscisic acid methyl ester, artemisinin, and its derivatives (Bhakuni et al. 2001).

2.1.4.13 Antifeedant Properties

Research studies have been conducted by implying various parameters of assessment of antifeedant activity for crude extracts of *A. annua*. Deterrency, growth regulatory effect and ovicidal potential strongly recommend it as a good antifeedant herb (Haghighian et al. 2008), as antihelminthes and anti-insecticidal agent (Khosravi et al. 2011; Vicidomini 2011). Some studies have reported that crude extracts of *A. annua* contain artemisinin and its derivatives which act as natural pesticide (WHO Monographs 2006; Huang et al. 2010; Weathers et al. 2011).

2.2 Conclusion

A. annua is ethanomedicinally important plant as its medicinal use has been well established in Chinese pharmacopeias since 168 BC *A. annua* has also obtained an important place among plant-based advanced therapeutics. Particularly against drug-resistant malaria, it has become a good hope for treatment, because it has

very low toxicity. Mefloquine is one of the antimalarial drug, but it is associated with multiple side effects. Recent several research studies have revealed that *A. annua* possess characteristic biological activities to cure various diseases. But their mechanisms of action at cellular and molecular level still need to be investigated. *A. annua* is a rich source of large number of biologically active phytoconstituents, and particularly, it is the only source of artemisinin. It possesses characteristic therapeutic potential against malaria, and besides antimalarial effects, it has various other biological activities such as anti-inflammatory, antibacterial, angiotensin converting enzyme inhibitory, cytokinin-like, and antitumor activities. Nowadays, there is increasing research focus toward investigation of its anticancer and antiviral effects particularly for HIV/AIDS. Therefore, mechanisms of action of the active phytoconstituents particularly artemisinin and their derivatives has become the emerging area of interest in the arena of scientific investigations. These research studies can validate the ethanomedicinal use of *A. annua* by local community on scientific bases. Therefore, *A. annua* is a strong alternate which can be widely explored and finally can lead toward drug development.

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