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Traditional application and modern pharmacological research of *Artemisia annua* L.

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ABSTRACT

As a Traditional Chinese Medicine, *Artemisia annua* L. (*A. annua*) has been used for the treatment of various diseases since ancient times, including intermittent fevers due to malaria, bone steaming and heat/fever arising from exhaustion, tuberculosis, lice, wounds, scabies, dysentery et al. With the discovery of artemisinin and its excellent anti-malarial activity, *A. annua* has received great attention. Recently, *A. annua* has been revealed to show inhibitory effects against parasites (*e.g. Plasmodium*, *Toxoplasma gondii*, *Leishmania*, *Acanthamoeba*, *Schistosoma*), viruses (*e.g.* hepatitis A virus, herpes simplex viruses 1 and 2, human immunodeficiency virus), fungi (*Candida*, *Malassezia*, *Saccharomyces* spp.) and bacteria (*Enterococcus*, *Streptococcus*, *Staphylococcus*, *Bacillus*, *Listeria*, *Haemophilus*, *Escherichia*, *Pseudomonas*, *Klebsiella*, *Acinetobacter*, *Salmonella*, *Yersinia* spp.). *A. annua* has also been reported to possess anti-inflammatory and anti-cancer actions and been employed for the treatment of osteoarthritis, leukemia, colon cancer, renal cell carcinoma, breast cancer, non-small cell lung cancer, prostate cancre and hepatoma. Besides, the immunoregulation, anti-adipogenic, anti-ulcerogenic, anti-asthmatic, anti-nociceptive and anti-osteoporotic activities of *A. annua* were also evaluated. Along these lines, this review summarizes the traditional application and modern pharmacological research of *A. annua*, providing novel insights of *A. annua* in the treatment of various diseases.

Contents

Abbreviations: AAE, *A. annua* extract; AALEO, essential oil from *A. annua* leaves; AAME, *A. annua* methanolic extract; ACT, artemisinin-based combination therapy; AS, artesunate; ASMCs, airway smooth muscle cells; CA16, cossac virus type A16; C/EBP, CCAAT/enhancer binding protein; CI, growth inhibitory concentration for 100% of the microorganisms; CL, cutaneous leishmaniasis; DLA, dried leaf *A. annua*; DLAe, dried leaf *A. annua* methylene chloride extracts; ECs, human umbilical vein endothelial cells; EMT, epithelial-mesenchymal transition; FabP4, fatty acid-binding protein 4; GIC_{50}, growth inhibitory concentration for 50% of the microorganisms; GLUT1, glucose transporter 1; HAV, Hepatitis A virus; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; HFD, high-fat diet; HFF, human foreskin fibroblasts; HIV, human immunodeficiency virus; HQG, polysaccharides isolated from *A. annua*; HSV, herpes simplex viruses; IZD, inhibition-zone diameter; JNK, Jun N-terminal kinase; Lac-FR, enriched sesquiterpene lactone fraction; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MIC, minimal inhibitory concentration; ML, mucosal leishmaniasis; MMC, minimal microbicidal concentration; MMP, matrix metalloproteinase; mTORC1, mechanistic target of rapamycin complex 1; NO, nitric oxide; NSCLC, non-small cell lung cancer; OVX, ovariectomized; PGE_2, prostaglandin E2; PI3K, phosphatidylinositol 3-kinase; pKAL, polyphenols from *A. annua*; PKM2, pyruvate kinase muscle isozyme M2; pPPARγ, eroximal proliferator-activated receptor-γ; PSA, prostate specific antigen; PTEN, phosphatase and tensin homolog; RANKL, receptor activator of nuclear factor kappa-B ligand; RCC, renal cell carcinoma; RSV, respiratory syncytial virus; SLF, sesquiterpene lactone fraction; TC, total cholesterol; TG, triglyceride; TLR, toll-like receptor; TRs, tracheal rings; ULI, ulcerative lesion index; VCAM-1, vascular cell adhesion molecule-1; VL, visceral leishmaniasis; WHO, World Health Organization.

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1. Introduction

Artemisia annua L. (*A. annua*), a plant belonging to the Asteraceae family, grows wild in Asia (mainly China, Japan and Korea) and it was introduced to Poland, Brazil, Spain, France, Italy, Romania, United States and Austria, where it became domesticated (Klayman, 1993). It has been used by Chinese herbalists for the treatment of various diseases since ancient times (Hsu, 2006; Liu, 2017). In 1967, a national research project against malaria was initiated in China. More than 380 herbal extracts were evaluated by Chinese scholar Tu Youyou for their anti-malarial activities and *A. annua* was found to be the most active herb (Tu, 2011). Then, in 1971, an endoperoxide sesquiterpene lactone named artemisinin was isolated and characterized as the active principle of *A. annua* against malaria. From then on, as the only commercial source of artemisinin, *A. annua* gained a widespread attention (de Ridder, van der Kooy, & Verpoorte, 2008).

Nowadays, there are still continuous efforts in delineating the mechanisms of action for anti-malaria activities of *A. annua* and artemisinin (Ding, Beck, & Raso, 2011; Wang et al., 2015). In the meantime, within the last few decades, *A. annua* has been investigated for its effects in various diseases, ranging from inflammatory, cancers to viral, bacterial and parasite-related infection (Alesaeidi & Miraj, 2016; Bilia, Santomauro, Sacco, Bergonzi, & Donato, 2014; Efferth, 2017). The extensive biological activities made *A. annua* a promising therapeutic to be widely used in clinical therapy. The aim of this review was to provide a comprehensive overview on the traditional application and the modern pharmacological research associated with *A. annua*, providing novel insights of *A. annua* in the treatment of various diseases.

2. Traditional application of *A. annua*

A. annua was first recorded in "52 Sickness Sides (Wu Shi Er Bing Fang)", a medical prescription excavated in the Mawangdui Han Tombs for the treatment of haemorrhoids. Application of *A. annua* for the treatment of fever and chills related to malarial was first mentioned by Hong Ge (284–365 CE) in "Handbook of Prescriptions for Emergencies". Nowadays, *A. annua* has been officially recognized as a medicinal plant and listed in Chinese Pharmacopeia. As recorded in ancient medical textbooks, *A. annua* was recommended for the treatment of intermittent fevers due to malaria, bone steaming and heat/fever arising from exhaustion, tuberculosis, lice, wounds, scabies, dysentery, acute convulsions related to pollution through contact with the dead, haemorrhoids, pain and swelling around tooth, pus in ear, rhinopolyp, and it also exerted eyesight improving, summer-heat relieving, hemostasis and analgesic activities (Fig. 1).

3. Biological activities of *A. annua*

3.1. Anti-parasitic activities of A. annua

3.1.1. Malaria parasites (Plasmodium)

Infection with malaria parasites may result in a wide variety of symptoms, ranging from absent or very mild symptoms to severe disease and even death. Malaria is still a leading cause of illness and death in several countries. The World Health Organization (WHO) recommends artemisinin-based combination therapy (ACT) for the treatment of uncomplicated malaria due to *Plasmodium falciparum*. Nowadays, a number of herbal remedies made of *A. annua* are available and suggested for the prevention and treatment of malaria. Even though WHO has cautioned against use of non-pharmaceutical *A. annua* plant material for the treatment or prevention of malaria, it is still believed that *A. annua* might offer an additional tool for the control of malaria due to the fact that *A. annua* could be cultivated and prepared with relative ease, especially in poor areas where access to effective anti-malarial drugs is precluded.

The anti-malarial activities of *A. annua* have been widely reported. In the clinical trial conducted by Ogwang et al., the protective effect of *A. annua* tea infusion was evaluated in 132 flower farm workers (Ogwang et al., 2012). *A. annua* tea infusion consumed once a week (2.5 dried leaves per infusion) significantly reduced the risk of suffering multiple episodes of malaria in nine months. In the clinical trial conducted by Mueller, 132 patients were involved and *A. annua* tea preparation rapidly improved the malaria symptoms and the cure rate was 74% (91% for quinine) after a seven-day treatment (Mueller et al., 2004). However, a higher rate of recrudescence was observed during follow-up. Similar cure rate of *A. annua* tea preparation was obtained in another clinical trial (Blanke et al., 2008). The minimum concentration of artemisinin required for growth inhibition of *Plasmodium falciparum* was reported to be 9 ng/mL and pharmacokinetic studies demonstrated that the plasma concentrations of artemisinin after intake of *A. annua* tea were higher than 9 ng/mL for at least four hours, indicating that tea preparation could provide sufficient artemisinin for clinical anti-malarial effects (Alin & Bjorkman, 1994; Rath et al., 2004). Taken together, using *A. annua* tea preparation for the treatment of malaria could be very encouraging, and further trials should consider the combinations of *A. annua* with other anti-malarial drugs or plants to reduce high rate of recrudescence (Willcox, Rasoanaivo, Sharma, & Bodeker, 2004).

Besides the *A. annua* tea preparation, powdered leaves of *A. annua* in capsules or tablets also exhibited excellent anti-malarial activities (Elfawal et al., 2012; Onimus, 2013; Wan, Zang, & Wang, 1992; Weathers, Towler, Hassanali, Lutgen, & Engeu, 2014). Pharmacokinetic studies revealed that the serum concentrations of artemisinin were 40-fold greater in mice fed with dried *A. annua* leaves than those fed with pure artemisinin (Cai, Zhang, Ji, & Xing, 2017; Weathers, Elfawal, Towler, Acquaah-Mensah, & Rich, 2014). Additionally, compared with pure artemisinin, 40-fold less artemisinin was required to obtain a comparable therapeutic effect (Weathers, Towler, et al., 2014). These results indicated that a complex matrix of chemicals existed in the leaves seems to be able to enhance both the bioavailability and efficacy of artemisinin. In the meanwhile, researchers recently found that treatment with the whole plant of *A. annua* could overcome existing resistance to artemisinin (Daddy et al., 2017; Elfawal, Towler, Reich, Weathers, & Rich, 2015). The long-term artificial selection of drug resistance in *Plasmodium chabaudi* parasites was investigated in mice (Elfawal et al., 2015). Stable resistance to artemisinin (100 mg/kg) was achieved at passage 16 and resistance to the whole plant (100 mg/kg) was not achieved even after 45 passages. In a case report, 18 patients who failed to respond to either ACT or *i.v.* artesunate were treated with DLA tablets, and all of them were recovered fully (Daddy et al., 2017). Even though there are still much work remains, the clear evidence of the efficacy of *A. annua* against malaria make it a promising therapy against malaria that is inexpensive and readily accessible.

3.1.2. Toxoplasma gondii

Human toxoplasmosis is a widely distributed infection caused by *Toxoplasma gondii*, an obligate intracellular protozoan. In immunocompetent individuals, most infections are asymptomatic; but in immunocompromised patients or during pregnancy, toxoplasma infection may lead to miscarriages or host death if not treated (Montoya &

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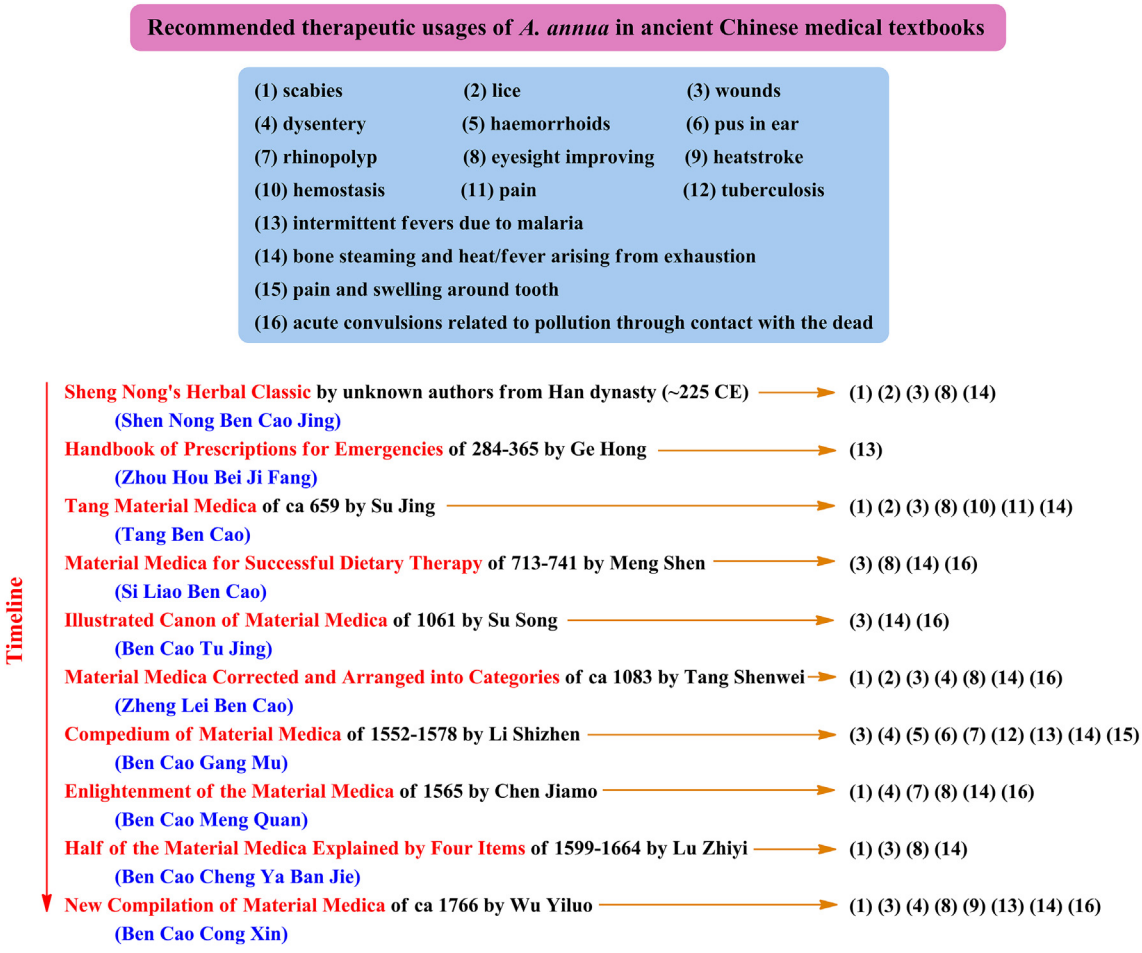

Fig. 1. Traditional applications of *A. annua* recorded in ancient Chinese medical textbooks.

Liesenfeld, 2004). Due to the fact that first-line medicine such as sulfadiazine or pyrimethamine is frequently not well tolerated and may cause many side effects, herbal derived medicines such as *A. annua* with low toxicity and low price have been widely investigated for their anti-toxoplasma activity (Rostkowska et al., 2016).

In the study conducted by Oliveira et al., the effect of *A. annua* infusion on *Toxoplasma gondii* infection was evaluated both *in vitro* and *in vivo* (de Oliveira et al., 2009). In the *in vitro* study, when *T. gondii* was treated with *A. annua* infusion before infection in human foreskin fibroblasts (HFF) cells, *A. annua* infusion showed an IC_{50} value of 95 μg/mL against *T. gondii*. However, when the treatment with *A. annua* infusion was conducted after the HFF cells were infected with *T. gondii*, the growth of the parasite could not be completely inhibited, reaching a maximum inhibition of 30%. In the *in vivo* study, subcutaneously administration of *A. annua* infusion at the dose of 10 mg/kg/day showed an effective control of infection. These results indicated that *A. annua* infusion affect more directly on the parasite than the infected cells. As we all know, artemisinin is an active component isolated from *A. annua* with excellent anti-malarial activity and it has been well-documented that artemisinin and its derivatives could inhibit *T. gondii* infection (Ho, Peh, Chan, & Wong, 2014). However, in the study conducted with artemisinin, contradictory result was obtained that pretreatment of host cells or *T. gondii* with artemisinin had no effect on *T. gondii* growth (Ke, Krug, Marr, & Berens, 1990). Additionally, in the investigation conducted by Rostkowska et al., the concentration of artemisinin in *A. annua* leaves was increased *via* the application of soil with silicate (400 kg/ha) (Rostkowska et al., 2016). However, they found that the infusion of *A. annua* grown in soil with or without silicate addition both decreased *T. gondii* proliferation in HeLa cells with similar dose-dependent manners. Thus, it was suggested that artemisinin was not the only active compound in *A. annua* possess anti-toxoplasma activity and the effectiveness of *A. annua* infusion may be partly due to other principles.

Since *T. gondii* infection can undergo transplacental transmission to the embryo during pregnancy. The effectiveness of *A. annua* infusion on the vertical transmission of *T. gondii* was evaluated in *Calomys callosus* infected with *T. gondii* ME49 stain (Costa et al., 2009). Results showed that *A. annua* could not inhibit the vertical transmission of *T. gondii*, although the number of parasites found in the placenta and fetal tissues was lower than in non-treated animals. Meanwhile, the observation of embryos in atrophy process in female animals treated with *A. annua* infusion warned us about the dangers of using *A. annua* in pregnant women.

3.1.3. Leishmania

Leishmaniasis is a disease caused by *Leishmania*, an intracellular protozoan. This disease manifests as three forms, namely cutaneous leishmaniasis (CL), mucosal leishmaniasis (ML), and visceral leishmaniasis (VL) (Burza, Croft, & Boelaert, 2018). Nowadays, first-line treatments such as sodium antimony et al. are unsatisfactory in terms of safety and efficacy, and alternatives are urgently needed.

Early in 1993, artemisinin and artemether were reported to be effective in experimental CL (D. M. Yang & Liew, 1993). Nowadays, it was proved that artemisinin exihibited antileishmanial activity against several species of *Leishmania via* inducing the apoptotic death in *Leishmania* (De Sarkar et al., 2019; Geroldinger et al., 2020; Sen et al., 2007; Sen,

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Saha, Sarkar, Ganguly, & Chatterjee, 2010). Meanwhile, 19 fluoro-artemisinin derivatives were synthesized and an amino derivative showed the strongest antileishmanial activity with an IC_{50} value of about 1 μM against three *Leishmania* lines. (Chollet, Crousse, Bories, Bonnet-Delpon, & Loiseau, 2008). Additionally, several novel drug delivery systems, such as nanoliposomal artemisinin and artemisinin-loaded nanoparticles were developed to increase the therapeutic efficacy of artemisinin and they both showed improved leishmanicidal activities compared with free artemisinin (Want et al., 2014; Want et al., 2017).

Due to the pronounced antileishmanial activity of artemisinin, the possibility of using *A. annua* for the treatment of leishmaniasis was investigated. In 2009, the anti-leishmanial activity of *A. annua* was confirmed in an *in vitro* study (Malebo et al., 2009). The *n*-hexane extract of the leaves of *A. annua* showed an IC_{50} value of 6.4 μg/mL against *Leishmania donovani*. In studies conducted by Islamuddin et al., *n*-hexane fractions of *A. annua* leaves and seeds could kill the promastigotes time-dependently at a concentration of 100 μg/mL *via* triggering programmed cell death in *Leishmania donovani* (Islamuddin et al., 2015; Islamuddin, Farooque, Dwarakanath, Sahal, & Afrin, 2012). Additionally, orally administration of *n*-hexane fractions of *A. annua* leaves and seeds to infected mice for ten consecutive days could significantly reduce the parasite burden in liver and spleen and decrease the spleen weight by switching on the Th1-based protective cell-mediated immunity with generation of memory (Islamuddin et al., 2015). The constituents in *n*-hexane extracts of *A. annua* leaves and seeds were identified as α-amyrinyl acetate, β-amyrine, cetin and artemisinin derivatives. In another study, essential oil from *A. annua* leaves (AALEO) with camphor (52.6%), β-caryophyllene (10.95%), 1,8-cineole (5.57%) and β-caryophyllene oxide (4.21%) as the most abundant compounds was prepared and evaluated for the leishmanicidal effect (Islamuddin et al., 2014). AALEO showed significant leishmanicidal effect against the promastigotes and intracellular amastigotes of *Leishmania donovani* with an IC_{50} of 14.63 and 7.3 μg/mL, respectively. After intraperitoneally administration of AALEO at the dose of 200 mg/kg to the infected mice, the parasite burden in liver and spleen was reduced by almost 90%. Meanwhile, in the above-mentioned studies, no cytotoxicity on macrophages or hepato- and nephrotoxicity on mice were observed for *A. annua* derived products. All these reports together suggested that *A. annua* is a promising herb for the treatment of VL.

Besides VL, the potential usefulness of *A. annua* for the treatment of CL was also evaluated (Mesa et al., 2017). Dried *A. annua* leaves powder were prepared into gelatin capsules and this capsule showed leishmanicidal activity on the intracellular amastigotes of *Leishmania (Viannia) panamensis* (EC_{50} = 48.07 μg/mL and EC_{90} = 82.2 μg/mL) without any cytotoxicity on murine macrophages. Additionally, five of six infected hamsters were cured by *A. annua* capsules (500 mg/kg/day, 30 days) and 2 CL patients were cured with the treatment of *A. annua* capsules (30 g, 45 days), without any side effects.

It was obvious that artemisinin was not the only component in *A. annua* possesses leishmanicidal activity. Camphor, β-caryophyllene and β-caryophyllene oxide might also contribute to its antileishmanial activity. In the study conducted by Soares et al., β-caryophyllene was reported to exhibit dose-dependent activity against intracellular amastigotes (IC_{50} = 6.4 μM) (Soares, Portella, Ramos, Siani, & Saraiva, 2013). Even though no direct evidence that camphor possesses leishmanicidal activity, however, a series of camphor hydrazine derivatives synthesized from camphor were reported to be effective (IC_{50} ranged from 21.78 to 58.23 μM) against *Leishmania amazonensis in vitro* (da Silva et al., 2020). To sum up, artemisinin together with campor and β-caryophyllene were the promising candidates for the development of novel leishmanicidal drugs.

3.1.4. Acanthamoeba

Acanthamoeba spp. is organism could cause infections such as amebic keratitis and granulomatous amebic encephalitis in humans. In the early 1990s, artemisinin and its derivatives, beta-arteether and sodium artesunic acid have been evaluated for their activities against primary amebic meningoencephalitis (S. Gupta, Dutta, & Vishwakarma, 1998; Gupta, Ghosh, Dutta, & Vishwakarma, 1995). Results showed that these compounds could slightly prolong the survival time of the model mice but they were not curative even at high doses (60–180 mg/kg for 5 days). Meanwhile, a recent *in vitro* study revealed that artemether showed amoebicidal activity against *Acanthamoe bacastellanii* in a time- and dose-dependent manner *via* inhibition of the serine biosynthesis pathway, which was important to amoeba survival (Deng et al., 2015). Based on these results, the possibility of using *A. annua* for the treatment of acanthamoebiasis was assessed in recent years (Derda et al., 2016;Wojtkowiak-Giera et al., 2018; Wojtkowiak-Giera et al., 2019). In the study conducted by Derda et al., water, alcohol and chloroform extracts of *A. annua* were confirmed to be effective against both trophozoites and cysts of *Acanthamoe bacastellanii* and the extracts could also prolong the survival time of the infected mice (Derda et al., 2016). Additionally, water extracts of *A. annua* was found to be effective for the treatment of infected mice *via* modulating the expression of components related with the immune system like Toll-like receptor 2 and 4 (Wojtkowiak-Giera et al., 2018; Wojtkowiak-Giera et al., 2019).

3.1.5. Schistosoma

Schistosomiasis is a parasitic disease caused by infection with *Schistosoma* spp. of parasitic flatworms. Since the early 1980s, artemisinin and its derivatives (artemether, artesunate, dihydroartemisinin et al.) have been reported to be effective against *Schistosoma* spp., notably larval parasites (Liu et al., 2014; Liu, Dong, & Jiang, 2012; Shuhua, Chollet, Weiss, Bergquist, & Tanner, 2000; Zhang et al., 2014). As the only commercial source of artemisinin, *A. annua* ethanolic extract (2.0 mg/mL) were able to kill all *Schistosoma mansoni* within 1 h *in vitro* (Ferreira, Peaden, & Keiser, 2011). Due to the fact that the contents of artemisinin and its derivatives in *A. annua* extracts were no more than 4%, it was suggested that other compounds in *A. annua* extracts may exhibit anthelmintic activity or synergistic effects of artemisinin. In a clinical trial, the effect of *A. annua* tea infusion on schistosomiasis was evaluated (Munyangi et al., 2018). After the patients were treated with *A. annua* tea infusion for 14 days, no schistosome eggs could be detected in feces. Compared with the current standard praziquantel treatment, *A. annua* tea infusion exhibited fewer side effects. Even though several critical issues existed in this clinical trial and further studies about the posology were still needed, *A. annua* tea infusion should be considered as an alternative to combat schistosomiasis (Argemi et al., 2019).

3.2. Anti-viral activities of A. annua

During the past few decades, the activity of artemisinin and its derivatives against viruses such as human herpes virus 6, herpes simplex viruses 1 and 2 (HSV1 and HSV2), Hepatitis B virus and bovine viral diarrhoea virus have been widely investigated and well documented (Blazquez et al., 2013; Efferth, 2018; Efferth et al., 2002; Efferth et al., 2008; Efferth et al., 2016; Romero et al., 2006). However, the anti-viral activities of *A. annua* were somehow ignored by researchers and only few investigations associated with Hepatitis A virus (HAV), HSV1 and HSV2, human immunodeficiency virus (HIV), respiratory syncytial virus (RSV) and cossac virus type A16 (CA16) were reported.

The HAV is a non-enveloped RNA virus which could cause acute hepatitis. *A. annua* could significantly reduce HAV titer by 2.33 logs when HAV was co-treated with 50 μg/mL *A. annua* extract (Seo et al., 2017). However, similar anti-viral activity was not observed when HAV was pre-treated with *A. annua* extract at the same concentration which indicated that *A. annua* extract may exert anti-viral activity *via* direct virucidal activity or hampering viral attachment to the host cells.

HSV1 and HSV2 are enveloped DNA viruses and HSV infections are responsible for several diseases ranging from Herpes Labialis to severe

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encephalitis. *A. annua* methanol extraction showed promising anti-viral activity against HSV1 in HeLa cells which was more effective than acyclovir at concentration of 3.125, 6.25, 12.5 and 25 μg/mL (Karamoddini, Emami, Ghannad, Sani, & Sahebkar, 2011). In another study, the aqueous extract of *A. annua* showed anti-viral activity against HSV2 in Vero cells which was as effective as acyclovir (Zhang, Tan, Pu, Liu, & He, 2003). However, the anti-viral activity against HSV2 was not observed for the petroleum ether, ethyl acetate and *n*-butanol extraction of *A. annua*. Further analysis of the *A. annua* aqueous extraction showed that the main constituents were carbohydrates and polyphenols. Based on these results, a condensed tannin with encouraging anti-HSV2 activity was isolated form the aqueous extract of *A. annua* (Zhang et al., 2004). Besides the HSV2, the condensed tannin also showed anti-hepatitis B virus (HBV) activity *via* the inhibition of hepatitis B e-antigen (HBeAg) secretion of HepG2 2.1.2 cells, a permanently cell line infected with HBV derived from HepG2 cells.

HIV is a fast-evolving virus could both impair and evade the host's immune system. An *in vitro* study revealed that *A. annua* tea infusion exhibited excellent anti-HIV activity with an IC_{50} of 2.0 μg/mL (Lubbe, Seibert, Klimkait, & van der Kooy, 2012). The contents of artemisinin in different *A. annua* tea samples were detected and interesting results were found that the most active sample had one of the lowest concentrations of artemisinin while the sample with the highest content of artemisinin showed one of the lowest activity. Meanwhile, pure artemisinin was inactive at 25 μg/mL and a similar level of anti-HIV activity was observed for *Artemisia afra*, a closely related species not containing any artemisnin. These results indicated that the role of artemisnin in the anti-HIV activity of *A. annua* was very limited. Additionally, it was found that *A. annua* methanol extraction showed a weak virus-cell infusion inhibitory activity (15.8%) and this might account for some of the action mechanisms of the anti-viral activity of *A. annua* (Chang & Woo, 2003).

In the study conducted by Lu et al., the volatile oil was extracted from *A. annua* and its hdyroxypropyl-β-cyclodextrin inclusion complex was prepared. They both showed anti-viral activities. The volatile oil of *A. annua* showed anti-viral activities against RSV and CA16 with an EC_{50} of 3.12 and 9.14 μg/mL while hdyroxypropyl-β-cyclodextrin inclusion complex of the volatile oil showed an EC_{50} of 0.28 and 0.59 μg/mL, respectively (Lu et al., 2018). The anti-viral activities of *A. annua* volatile oil were significantly increased after being prepared as inclusion complex.

As we all know, the inhibition of viral enzymes, viral replication, and viral protein synthesis *via* interaction with cellular molecules may account for the anti-viral mechanisms of herbal extracts (Jassim & Naji, 2003). Several researchers believed that antioxidant components in *A. annua* such as flavonoids may be responsible for its anti-viral activities (Chen, Plumb, Bennett, & Bao, 2005). But in Seo's study, the antioxidant activity of herb extracts (including *A. annua*) was not proportional to their anti-HAV activity (Seo et al., 2017). Other researchers believed that the artemisinin and its derivatives may be responsible for the anti-viral activity of *A. annua* due to the fact that artemisinin and its derivatives showed excellent anti-viral activities. However, in Lubbe's study, it was proved that the anti-HIV activity of *A. annua* was not related to artemisinin (Lubbe et al., 2012). Thus, the action mechanisms of the anti-viral activity of *A. annua* were still unclear and further studies were still needed.

3.3. Anti-fungal and anti-bacterial activities of A. annua

Recently, the attention of investigators regarding *A. annua* has been focused on its anti-fungal and anti-bacterial activities and the most widely investigated ones were *A. annua* essential oils. Various fungi and bacteria have been investigated including gram-positive bacteria (*Enterococcus*, *Streptococcus*, *Staphylococcus*, *Bacillus*, *Listeria* spp.), gram-negative bacteria (*Haemophilus*, *Escherichia*, *Pseudomonas*, *Klebsiella*, *Acinetobacter*, *Salmonella*, *Yersinia* spp.) and fungi (*Candida*, *Malassezia*, *Saccharomyces* spp.). Table 1 summarized the anti-fungal and anti-bacterial activities of *A. annua* essential oil. As it had been reported, French oil showed no anti-bacterial activity against *Escherichia coli* and *Staphylococcus aureus*, while Romanian oil, Italian oil and Chinese oil all showed anti-bacterial activities towards these two stains. This contradictory result may be caused by the differences of the stains and the chemical compositions of the oil used in these studies. As we can see, chemical profiles of the essential oil varied a lot, and camphor, artemisia ketone and 1,8-cineole were the main components in oil from the aerial parts of *A. annua*. For essential oil obtained from the seeds of *A. annua*, *trans*-3(10)-caren-4-ol was the most abundant component and camphor was not detected (Habibi, Ghanian, Ghasemi, & Yousefi, 2013). Additionally, the vapor-phase of the oil and the spike oil exhibited stronger anti-microbial activity since the contents of terpenoids in them were higher than that in the total oil and the stem oil, respectively (Li, Hu, Zheng, Zhu, & Liu, 2011; Santomauro et al., 2016; Santomauro et al., 2018). The main isolated constituents were also widely studied and they showed remarkable anti-microbial activities (Bilia et al., 2014; Donato, Santomauro, Bilia, Flamini, & Sacco, 2015; Marinas et al., 2015). However, the total oil showed stronger anti-microbial activity, suggesting that the anti-microbial activity of essential oil was at least in part due to synergistic effects of the components and the anti-microbial activity of the main components might be modulated by other minor constituents. Besides the essential oil of *A. annua*, the leaves powder extraction and the crude extraction of the whole plant also showed anti-microbial activities, making *A. annua* a promising source of new anti-microbial agents (Gupta, Dutta, Pant, Joshi, & Lohar, 2009; Pawar, Nirgude, & Shinde, 2015). However, *in vivo* studies assessing the anti-microbial activities of *A. annua* is still unavailable and the strengths and weaknesses of *A. annua* compared with the existing anti-microbial agents are not clear. Further investigations are required to fully evaluate the potential of anti-microbial activities of *A. annua* for clinical use.

3.4. Anti-inflammatory activities of A. annua

The anti-inflammatory activities of artemisinins have been widely investigated in various inflammatory disease models, such as autoimmune diseases, allergic inflammation and septic inflammation (Ho et al., 2014). Mechanism studies revealed that their anti-inflammatory activities were attributed to the inhibition of the mitogen-activated protein kinase (MAPK), PI3K/Akt signaling cascade, NF-κB activation and Toll-like receptor 4 (TLR4) and TLR9 expressions (Wang et al., 2017). Except artemisinins, the anti-inflammatory activity of *A. annua* was not that well-documented with only few studies available.

A. annua was firstly reported to possess anti-inflammatory properties in 1993 in mouse and rat inflammatory models caused with yeast powder (injection under the aponeurosis), dimethylbenzene (auricle smear method) and egg white (injection under the aponeurosis) respectively, when orally administration of *A. annua* water extraction (15, 30 and 60 g/kg for 4 or 6 consecutive days) markedly inhibited inflammatory reactions (Huang et al., 1993). The anti-inflammatory properties of four-artemisinin-containing extracts (water, methanol, ethanol and acetone extracts) of *A. annua* were evaluated in an *in vitro* study (Kim et al., 2015). Acetone extract (100 μg/mL), which contained the highest content of artemisinin, showed the greatest inhibitory effect on lipopolysaccharide (LPS)-activated nitric oxide (NO), prostaglandin E_2 (PGE2), and pro-inflammatory cytokine (IL-1β, IL-6, and IL-10) production in RAW 264.7 macrophages. Similar results were gained in Chougouo's study (Chougouo et al., 2016). Ethanol extract of *A. annua* at the concentration of 6.25, 12.5, 25 and 50 μg/mL, and five isolated components (artemisinin, scopoletin, chrysosplenetin, eupatin and 3-*O*-β-D-glucopyranoside of sitosterol) at the concentration of 0.5, 2, 5 and 20 μg/mL all inhibited the production of NO in LPS-induced RAW 264.7 macrophages. Another *in vitro* study assayed the anti-inflammatory potential of *A. annua* tea infusions on intestinal

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Table 1
Anti-fungi and anti-bacterial activities of *A. annua* essential oil.

A. annua essential oil	Effects	Chemical composition	Notes	References
French oil	*Candida albicans*: GIC_{50} = 0.1 mg/mL, CI = 0.2 mg/mL (Nystaine, GIC_{50} = 0.003 mg/mL, CI = 0.006 mg/mL). *Saccharomyces cerevisiae*: GIC_{50} = 0.1 mg/mL, CI = 0.2 mg/mL (Nystaine, GIC_{50} = 0.003 mg/mL, CI = 0.006 mg/mL). *Enterococcus hirae*: GIC_{50} = 0.05 mg/mL, CI = 0.1 mg/mL (Penicilline G, GIC_{50} = 0.0003 mg/mL, CI = 0.0008 mg/mL).	Camphor (44%), germacrene D (16%), trans-pinocarveol (11%), β-selinene (9%), β-caryophyllene (9%), and artemisia ketone (3%).	The essential oil showed no antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*	(Juteau, Masotti, Bessiere, Dherbomez, & Viano, 2002)
Bosnian oil	*Candida krusei*: IZD = 30 mm (Essential oil = 10 mg/mL). *Enterococcus faecalis*: IZD = 27 mm (Essential oil = 10 mg/mL). *Streptococcus pneumoniae*: IZD = 50 mm (Essential oil = 10 mg/mL). *Haemophilus influenza*: IZD ≫ 60 mm (Essential oil = 10 mg/mL). Ampicillin (10.0 mg/mL) was used as positive control and no remarkable inhibition zones were observed.	Artemisia ketone (30.7%), camphor (15.8%), and artemisia alcohol (6.5%).	Antioxidant activities of the essential oil were also assayed and the essential oil showed a comparable antioxidant activity with thymol.	(Ćavar, Maksimović, Vidic, & Parić, 2012)
Romanian oil	*Staphylococcus aureus*: For ATCC 6538 stain, MIC = 1.02 mg/mL, MMC = 1.02 mg/mL. For MRSA 1263 stain, MIC = 4.08 mg/mL, MMC = 4.08 mg/mL. *Bacillus subtilis*: For 12488 stain, MIC = 2.04 mg/mL, MMC = 2.04 mg/mL. For ATCC 6683 stain, MIC = 2.04 mg/mL, MMC = 4.08 mg/mL. *Enterococcus faecalis*: MIC = 0.51 mg/mL, MMC = 8.17 mg/mL. *Pseudomonas aeruginosa*: For ATCC 27853 stain, MIC = 8.17 mg/mL, MMC = 32.7 mg/mL. For 134,202 stain, MIC = 8.17 mg/mL, MMC = 16.3 mg/mL. For 326 stain, MIC = 8.17 mg/mL, MMC = 32.7 mg/mL. *Escherichia coli*: For ATCC 13202 stain, MIC = 16.3 mg/mL, MMC = 16.3 mg/mL. For $O_{126}B_{16}$stain, MIC = 16.3 mg/mL, MMC = 16.3 mg/mL. *Klebsiella pneumoniae*: For ATCC 134202 stain, MIC = 16.3 mg/mL, MMC = 16.3 mg/mL. For 11 stain, MIC = 2.04 mg/mL, MMC = 32.7 mg/mL. *Acinetobacter baumannii*: MIC = 8.17 mg/mL, MMC = 8.17 mg/mL. *Candida famata*: For 945 stain, MIC = 2.04 mg/mL, MMC = 2.04 mg/mL. For CMGBy.14 stain, MIC = 2.04 mg/mL, MMC = 4.08 mg/mL. *Candida utilis*: MIC = 4.08 mg/mL, MMC = 4.08 mg/mL. *Candida albicans*: For 393 stain, MIC = 2.04 mg/mL, MMC = 4.08 mg/mL. For ATCC 101103 stain, MIC = 2.04 mg/mL, MMC = 4.08 mg/mL.	Camphor (17.74%), α-pinene (9.66%), germacrene D (7.55%), 1,8-cineole (7.24%), trans-β-caryophyllene (7.02%), and artemisia ketone (6.26%).	The anti-microbial activities of the main active compounds were also investigated and the most active component was camphor. The anti-microbial activity of essential oil was at least in part due to synergistic effects of the components.	(Marinas et al., 2015)
Italian oil	*Candida* spp. includes *C. krusei*, *C. parapsilosis*, *C. dubliniensis*, *C. glabrata*, *C. norvegensis*, *C. tropicalis*, and *C. albicans*. For the liquid-phase of the oil: average MIC = 11.88 μL/mL. For the vapor-phase of the oil: the growth of all *Candida* strains was inhibited at a concentration of 2.13 $\mu L/cm^3$.	Artemisia ketone (22%), 1,8 cineole (19%), camphor (17%), artemisia alcohol (5.9%), α-pinene (5.7%), and pinocarvone (3.0%).	The anti-fungi activity of *A. annua* essential oil was higher in the vapor than in the liquid phase. *C. albicans* and *C. dubliniensis* were the most susceptible while *C. parapsilosis* was the most resistant.	(Santomauro et al., 2016)
Italian oil	*Malassezia* spp. includes *M. furfur*, *M. sloffiae*, *M. sympodialis*, *M. pachydermatis*, and *M. globosa*. For the liquid-phase of the oil: MMC ranged from 0.78 μL/mL to 3.125 μL/mL and all strains were inhibited when treated with amphoterichin B (12.5 μg/mL). For the vapor-phase of the oil: the concentrations required to totally inhibit the growth of the strains ranges from 0.066 to 1.06 $\mu L/cm^3$ of air.	For liquid-phase of the oil: camphor (25.2%), 1,8-cineole (20%) and artemisia ketone (12.5%). For the vapor-phase of the oil: α-Pinene (22.8%), 1,8-cineole (22.1%) and camphene (12.9%).	The anti-microbial activity of the vapor phase of oil was stronger than that of the total oil.	(Santomauro et al., 2018)
Italian oil	*Escherichia coli* O157: MMC = 17.6 mg/mL. *Salmonella Enteritidis*: MMC = 0.18 mg/mL.	Artemisia ketone (24%), camphor (17.7%) and 1,8-cineole (16.1%).	Artemisia ketone, 1,8-cineole and camphor, the three main constituents of *A. annua*	(Donato et al., 2015)

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Table 1 (*continued*)

A. annua essential oil	Effects	Chemical composition	Notes	References
	Salmonella Typhi: For ATCC 19430 stain, MMC = 11.8 mg/mL. For CIP 6062 stain, MMC = 17.6 mg/mL. *Yersinia enterocolitica*: For YeDHS11 strain, MMC = 23.5 mg/mL. For YeDHS17 strain, MMC = 0.18 mg/mL. *Listeria monocytogenes*: MMC = 17.6 mg/mL.		essential oil, were also tested for their anti-bacterial activities and they all showed lower activity than the total oil.	
Iranian oil from seeds of *A. annua*	*Staphylococcus aureus*: IZD = 14 mm (Essential oil = 10 μL) *Bacillus subtilis*: IZD = 14 mm (Essential oil = 10 μL) *Enterococcus faecalis*: IZD = 19 mm (Essential oil = 10 μL) *Escherichia coli*: IZD = 20 mm (Essential oil = 10 μL)	*Trans*-3(10)-caren-4-ol (22.3%), artemisia ketone (18.6%), 1,8-cineole (14.9%),δ-selinene (13.0%) and α-pinene (8.2%)	Trans-3(10)-caren-4-ol was the most abundant component and camphor, which was the dominant compound in all the reported oil was not detected in this seed's oil.	(Habibi et al., 2013)
Chinese oil	*Staphyloccocus aureus*: $MIC_{stem\ oil}$ = 15.6 μg/mL, MIC_{spike} = 31.3 μg/mL. *Escherichia coli*: $MIC_{stem\ oil}$ = 31.3 μg/mL, MIC_{spike} = 31.3 μg/mL. *Bacillus subtilis*: $MIC_{stem\ oil}$ = 7.81 μg/mL, MIC_{spike} = 7.81 μg/mL. *Bacillus thuringiensis*: $MIC_{stem\ oil}$ = 31.3 μg/mL, MIC_{spike} = 31.3 μg/mL.	Methyl cinnamate (9.70%), phenylacetic acid (4.88%), isobornyl acetate (3.85%), β-guaiene (3.51%) and trans-ocimene (3.50%).	The content of terpenoids in the spike oil was higher than that in the stem oil. The anti-microbial activity of the spike oil was stronger than that of the stem oil.	(Li et al., 2011)

GIC_{50}: growth inhibitory concentration for 50% of the microorganisms; CI: growth inhibitory concentration for 100% of the microorganisms; IZD: inhibition-zone diameter; MIC: minimal inhibitory concentration; MMC: minimal microbicidal concentration.

inflammation using Caco-2 cells at 3300 μg/mL (Melillo de Magalhães et al., 2012). In normal Caco-2 cells, no anti-inflammatory effect was observed, while in inflamed Caco-2 cells (stimulated by a cocktail of pro-inflammatory), *A. annua* tea infusion significantly reduce the secretion of IL-6 and IL-8. This study also uncovered that the anti-inflammatory activities of *A. annua* on inflamed intestinal epithelium were not related to the presence of artemisinin, but could be partly attributed to rosmarinic acid, a main phenolic component identified in *A. annua* extract. Studies have also revealed that casticin and chrysosplenol D, two flavonoids isolated from *A. annua* exhibited pronounced anti-inflammatory effects in mouse models of local and systemic inflammation, as well as in cultured mouse macrophages (Li et al., 2015). Topically treatment of casticin (0.5, 1 and 1.5 μmol/cm^2) and chrysosplenol D (1 and 1.5 μmol/cm^2) reduced croton oil-induced edema in mice. Meanwhile, pretreatment of mice with casticin (0.07, 0.13 and 0.27 mmol/kg) and chrysosplenol D (0.07, 0.14 and 0.28 mmol/kg) significantly reduced the systemic immune response to LPS through suppressing the expression of inflammatory mediators *via* the regulation of NF-κB and c-JUN. Taken together, these findings strongly support a therapeutic role for *A. annua* in the treatment of inflammatory disease, even though long-term (4 or 6 consecutive days) and high dose (15–60 g/kg *in vivo*, and 100 or 3000 μg/mL *in vitro*) administration of *A. annua* might be required. Artemisinin, scopoletin, chrysosplenetin, eupatin, 3-*O*-β-D-glucopyranoside of sitosterol, rosmarinic acid, casticin and chrysosplenol D are the major components exhibit anti-inflammatory activity.

The anti-inflammatory actions of *A. annua* have also been reported in humans. In a pilot randomized, placebo-controlled clinical trial conducted on forty-two subjects with osteoarthritis of the hip and knee, 150 mg *A. annua* extract twice daily reduced in pain, stiffness and functional limitation in patients (Stebbings, Beattie, McNamara, & Hunt, 2016). Notably, 150 mg *A. annua* extract twice daily appeared to be safe and well tolerated with no adverse events observed. However, when patients were treated with high dose of *A. annua* extract (300 mg twice daily), 28.6% of them showed adverse events like upper gastrointestinal symptoms and no statistically significant therapeutic effects could be obtained compared with placebo. In another clinical trial, the effect of the complementary use of *A. annua* plus disease-modifying antirheumatic drugs (leflunomide and methotrexate) was evaluated in patients with active rheumatoid arthritis (Yang et al., 2017). 159 patients with active rheumatoid arthritis were assigned to control group (80 cases, treated with leflunomide and methotrexate) and *A. annua* extract group (79 cases, treated with leflunomide, methotrexate plus *A. annua* extract at a dose of 30 g/day). At 12 weeks post-treatment, no overall efficacy was seen, however, significantly improvement of measures of acute inflammation like pain score, number of painful joints, erythrocyte sedimentation rate together with better overall efficacy were observed at 24 and 48 weeks post-treatment. These promising results suggested the complementary treatment of *A. annua* could improve the medium- and long-term therapeutic effect of rheumatoid arthritis.

3.5. Anti-cancer activities of *A. annua*

Since the late 1990s, the anti-cancer properties of artemisinin and its derivatives (artesunate and dihydroartemisinin) have been evaluated by various groups (Bhaw-Luximon & Jhurry, 2017). It has been reported that artemisinin and its derivatives exert anti-cancer effect *via* inducing cancer cell growth cycle arrest, promoting apoptosis, and inhibiting the angiogenesis and tissue invasion of tumor (Ho et al., 2014). Besides artemisinin, a variety of *A. annua* related products, including isolated polysaccharides, polyphenols, fractions, and different *A. annua* solvent extracts were also evaluated for their anti-cancer activities against various cancers (Table 2). Taken together, *A. annua* exhibited anti-cancer effects *via* inducing G1 and G2/M cell cycle arrest, reducing mitochondrial membrane potential, modulating PTEN/PDK1/Akt/p53 signal pathways, inhibiting cell glucose metabolism, reducing VCAM-1 expression and inhibiting MMP-2, MMP-9 and EMT (Fig. 2). The anti-cancer activities of *A. annua* were not only reported in cell and animal studies, it was also reported in human studies. In a case report, the activity of *A. annua* capsules in a patient with progressive and metastasized prostate carcinoma was described (Michaelsen, Saeed, Schwarzkopf, & Efferth, 2015). Long-term treatment with *A. annua* capsules after short-term treatment with bacalitumide resulted in impressive decrease of tumor marker prostate specific antigen (PSA) and tumor regression. Unfortunately, resistance phenomena occurred seven months later and the

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Table 2
Anti-cancer activities of *A. annua*.

Compounds	Cancers	Remarks	Subjects	Dose	Effects	Notes	References
Polysaccharides isolated from *A. annua* (HQG)	Hepatoma	*In vivo*	Tumor xenograft mice induced by injection of mouse hepatoma H22 cells	12.5, 25, 50 and 100 mg/kg (i.g.)	HQG inhibited tumor growth in a dose-dependent manner. HQG (50 mg/kg) markedly increase the cell apoptosis rate, the numbers of $CD4^+$ and $CD8^+$ T lymphocytes, the ratio of $CD4^+$/$CD8^+$, and the secretion of IFN-γ and IL-4.	HQG exerted anti-hepatoma activity by facilitation cell apoptosis and immune defence.	(Chen, Chen, Wang, & Liu, 2013)
		In vitro	Human hepatoma cell line 7402	50 μg/mL	HQG treatment decreased the mitochondrial membrane potential.		
Polyphenols from *A. annua* (pKAL)	Breast cancer	*In vitro*	Human breast cancer cell line MDA-MB-231	1, 10, 50 and 100 μg/mL	pKAL inhibited cell viability of MDA-MB-231 cells in a dose-dependent manner, but not that of human umbilical vein endothelial cells (ECs) until 50 μg/mL.	pKAL exerted anti-metastasis activity by suppression of VCAM-1 expression and invasion by inhibition of EMT.	(Ko et al., 2016)
				1, 10 and 30 μg/mL	pKAL (10 and 30 μg/mL) inhibited the adhesion of MDA-MB-231 cells to ECs through reducing VCAM-1 expression of MDA-MB-231 and CEs. pKAL (10 and 30 μg/mL) inhibited TNF-activated MDA-MB-231 cells invasion through inhibition of MMP-2 and MMP-9 and epithelial-mesenchymal transition (EMT).		
A. annua extract (AAE)	Colon cancer	*In vitro*	HCT116 colon cancer cells	20–100 μg/mL	AAE inhibited cell viability of HCT116 cells, but not that of normal human fibroblast cells.	AAE induced apoptosis through PTEN/PDK1/Akt/p53 signal pathway and mitochondria-mediatd apoptotic proteins.	(Kim et al., 2017)
				30, 40 and 60 μg/mL	AAE increased the levels of PTEN, p53 and mitochondria-mediated apoptotic proteins Bak, Bax and PUMA in a dose-dependent manner. AAE reduced mitochondria membrane potential and the cell survival proteins such as p-PDK1, p-Akt, p-MDM2, Bcl-2 and pro-caspase-3.		
				40 μg/mL	AAE regulated cytochrome *c* translocation to the cytoplasm and Bax translocation to the mitochondrial membrane.		
		In vivo	Tumor xenograft mice induced by injection of HCT116 human colon cancer cells	20 and 40 mg/kg/day	AAE treatment significantly reduced the tumor volume and increased PTEN and p53 expression in tumor xenograft mice. AAE induced apoptosis by regulating the phosphorylation of PDK1 and Akt through the PTEN/p53-independent pathway.		
Apartially purified material of *A. annua* (MC-4)	Advanced renal cell carcinoma (RCC)	*In vitro*	Human RCC cell lines Caki-1 and 786-O	0–320 μg/mL	MC-4 inhibited cell viability of RCC cells in a dose-dependent manner (Caki-1: IC_{50} = 95 μg/mL, 786-O: IC_{50} = 124 μg/mL).	Combination of MC-4 and everolimus showed synergistic anti-cancer and anti-metastatic effects *via* modulating PI3K/Akt/PKM2 and mTORC1 pathways. Clinical application of MC-4 together with mTOR inhibitors was recommended for metastatic RCC patients.	(Son et al., 2018)
				25, 50 and 100 μg/mL	MC-4 induced potent G2/M cell cycle arrest of RCC cells by upregulating $p27^{Kip1}$ and phospho-p53 and downregulating cyclin B1 and CDK1/4. MC-4 induced RCC cells autophagy *via* inhibition of cell glucose metabolism modulated by Akt/PKM2, with upregulated PTEN and reduced phosphorylation of Akt, PKM2, and GLUT1 expression observed. MC-4 (100 μg/mL) combined with everolimus (1 μM), a mTORC1 inhibitor, displayed synergistic anti-cancer activities.		

Table 2 (*continued*)

Compounds	Cancers	Remarks	Subjects	Dose	Effects	Notes	References
		In vivo	Tumor xenograft mice induced by injection of RCC cells	200 mg/kg (i.g.)	MC-4 treatment significantly reduced the tumor volume. Combination treatment of MC-4 (200 mg/kg) and everolimus (10 mg/kg) reduced the lung metastatic foci. Combination treatment showed synergistic effect in increased autophagic cell death.		
A. annua methanolic extract (AAME)	Acute lymphoblastic leukemia	*In vitro*	Acute lymphoblastic leukemia cell lines Nalm-6 and Reh	10–90 μg/mL	AAME exerted time- and dose-dependent cytotoxic effects on Nalm-6 and Reh cells. AAME (40 μg/mL) increased the mRNA expression level of caspase 3 and Bax. Combination treatment of AAME augmented the anti-cancer effect of vincristine.	AAME exhibited cytotoxicity effect and was able to enhance the anti-cancer effect of vincristine.	(Mashati, Esmaeili, Dehghan-Nayeri, Darvishi, & Gharehbaghian, 2019)
Powdered dried leaf *A. annua* (DLA), dried leaf *A. annua* methylene chloride extracts (DLAe) and artesunate (AS)	Non-small cell lung cancer (NSCLC)	*In vitro*	NSCLC cell lines A549, H1299 and PC9	0–200 μM for DLAe and AS	DLAe and AS suppressed A549, H1299 and PC9 cell viability at least partly *via* inducing DNA damage (with no inhibition of normal human dermal fibroblasts). DLAe and AS induced G2/M cell cycle arrest in PC9 and H1299 cells, and DLAe induced G1 cell cycle arrest in A549 cells. DLAe inhibit migratory ability of PC9 and A549. DLAe and AS induced the activation of caspase-3 and 9 in all three cells. Caspase-8 was activated by DLAe and AS in A549 and PC9 cells, but not in H1299 cells.	DLA showed anti-cancer activity against NSCLC *via* slowing proliferation, stimulating cell cycle arrest and inducing apoptosis. DLA inhibited A549 and PC9 induced tumor growth, while AS only inhibited A549 tumor growth.	(Rassias & Weathers, 2019)
		In vivo	Tumor xenograft mice induced by injection of A549 and PC9 cells	Equaled to 85 mg/kg of artemisinin for DLA or AS (i.g.)	In A549 xenografts, treatment of DLA or AS significantly inhibited the tumor growth. In PC9 xenografts, DLA inhibited tumor growth by ~50%, however, AS showed no effects.		

HQG: polysaccharides isolated from *A. annua*; pKAL: polyphenols from *A. annua*; ECs: human umbilical vein endothelial cells; VCAM-1: vascular cell adhesion molecule-1; MMP: matrix metalloproteinase; EMT: epithelial-mesenchymal transition; MC-4: a partially purified material of *A. annua*; RCC: renal cell carcinoma; mTORC1: mechanistic target of rapamycin complex 1; PKM2: pyruvate kinase muscle isozyme M2; PI3K: phosphatidylinositol 3-kinase; GLUT1: glucose transporter 1; PTEN: phosphatase and tensin homolog; AAE: *A. annua* extract; AAME: *A. annua* methanolic extract; NSCLC: non-small cell lung cancer; DLA: powdered dried leaf *Artemisia annua*; DLAe: dried leaf *Artemisia annua* methylene chloride extracts; AS: artesunate.

sensitivity of the tumor towards *A. annua* was decreased. Even though only one patient was involved and resistance phenomena occurred, the observed promising efficacy of *A. annua* made it still necessary for clinical trials to be conducted to evaluate the clinical benefit of *A. annua* in prostate cancer.

As we can see from the results in Table 2, some of the doses of *A. annua* related products used in *in vitro* studies were quite high. For example, 20–100 μg/mL of AAE was used to inhibit the cell viability of HCT116 cells and the dose of DLAe to inhibit cell viability of NSCLC cell lines was 0–200 μM. IC_{50} values of MC-4 for human RCC cell lines Caki-1 and 786-O were 95 μg/mL and 124 μg/mL, respectively. These *in vitro* results indicated that long-term administration of high dose of *A. annua* might be required for clinical application. Even though artemisinin is known to be well tolerated for the treatment of malaria, however, the tolerability of *A. annua* in cancer patients is still needed to be evaluated. This raises a question of whether *A. annua* is suitable for clinical application for the treatment of cancer. Meanwhile, combination use of *A. annua* enhanced the efficacy of everolimus and vincristine. It was believed that artemisinin was more efficient in terms of targeting cancer cells due to their high intracellular iron levels, which is essential for rapid cell division and proliferation. Hence, combination *A. annua* with synthetic chemodrugs to enhance the latter's efficacy might be a future direction for the development of *A. annua*.

Nowadays, it is widely accepted that artemisinin is not the only anti-cancer activity component in *A. annua*. In fact, early in 1994, quercetagetin 6,7,3′,4′-tetramethyl ether, a flavonoid component, was reported to exert cytotoxicity against P-388, A-549, HT-29, MCF-7 and KB tumor cells (Zheng, 1994). In the study conducted by Lang et al., the anti-cancer activity of an extract of an artemisinin-deficient *A. annua* preparation against breast cancer was investigated both *in vitro* and *in vivo* (Lang et al., 2019). This extract, with chrysosplenol D, arteannuin B, and casticin as the most abundant ingredients, significantly inhibited the cell proliferation, induced apoptosis and decreased tumor growth, proved that *A. annua* contained multiple components possess potential anti-cancer activity. Meanwhile, in the study conducted by Rassias et al., the anti-cancer activities of dried leaf *A. annua* (DLA) and artesunate against non-small cell lung cancer (NSCLC) were assessed at the same dose of equivalent molar amount of artemisinin (Rassias & Weathers, 2019). Results showed that DLA was more effective than artesunate in inhibiting tumor growth in tumor xenograft mice. Several reasons might account for this: (1) other components exist in DLA might increase the bioavailability of artemisinin *via*

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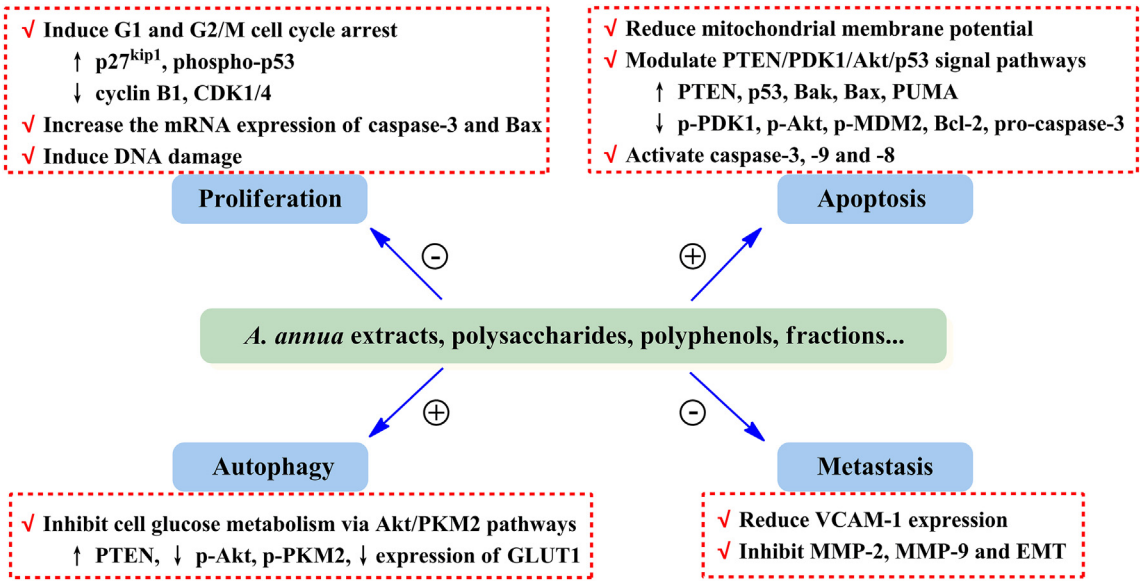

Fig. 2. Overview of the mechanisms of anti-cancer action of *A. annua*. + indicates activation and – indicates inhibition. ↑ indicates upregulation and ↓ indicates downregulation. Abbreviations are listed in Table 2.

improving its intestinal permeability or reducing its first-past metabolism. (2) Other components might also exert anti-cancer activities. (3) DLA exhibited anti-cancer efficacy by the synergic action with multiple chemical components.

Casticin and chrysosplenol D are the two flavonoids components proved to possess anti-cancer activities. Casticin is a polymethoxy flavone commonly found in many herbal plants and the content of it in *A. annua* is 1.07 ± 0.23 mg/g (Fu, Yu, Wang, & Qiu, 2020). Numerous *in vitro* studies affirmed that casticin showed antiproliferative and apoptotic activities against many cancer cell lines, including breast, bladder, colon, lung, ovarian cancers and others, with an IC_{50} value ranged from 0.4 to 28.7 μM (Ramchandani, Naz, Lee, Khan, & Ahn, 2020). Mechanism studies revealed that casticin could induce cell apoptosis *via* various signaling pathways including PI3K/Akt, STAT3, NF-κB and FOXO3a/FoxM1 (Ramchandani et al., 2020). Additionally, the anti-cancer activity of casticin was also evaluated *in vivo* (Lai et al., 2019;Qiao et al., 2019; Shiue et al., 2016). Intraperitoneally administration of casticin (2 and 10 mg/kg) significantly inhibited the tumor growth in both A375.S2 human melanoma cell and ECA-109 human esophageal cell tumor xenograft mice models (Qiao et al., 2019; Shiue et al., 2016). Chrysosplenol D is another flavonoids and the content of it in *A. annua* is about 0.64 ± 0.14 mg/g (Fu et al., 2020). In the study conducted by Lang et al., chrysosplenol D was proved to be able to inhibit the viability of several cell lines, namely, breast cancer cell lines MDA-MB-231 (IC_{50} = 11.6 μM) and MCF7 (IC_{50} = 36.4 μM), NSCLC cell line A549 (IC_{50} = 7.3 μM), pancreatic cancer cell line MIA PaCa-2 (IC_{50} = 35.6 μM) and prostate carcinoma cell line PC-3 (IC_{50} = 40.8 μM) (Lang et al., 2020). Even though the therapeutic uses of casticin and chrysosplenol D were only reported in preclinical studies and the safety and efficacy of them have not been evaluated by clinical trials yet, the promising anti-cancer activities of them opened new perspectives for the development of them as potential anti-cancer therapeutics.

3.6. Other activities

A. annua was also reported to possess other pharmacological activities including immunoregulation, anti-adipogenic, anti-ulcerogenic, anti-asthmatic, anti-nociceptive and anti-osteoporotic activities (Fig. 3). Detailed information was summarized in this section.

3.6.1. Immunoregulation activities

Due to the fact that *A. annua* was widely used for the treatment of autoimmune diseases like rheumatoid arthritisin ancient China, it was anticipated that *A. annua* should possess immunoregulation activities. In Zhang's study, the immunosuppressive effects of *A. annua* was evaluated (Zhang & Sun, 2009). Ethanol extract of *A. annua* at concentrations of 1–100 μg/mL significantly reduced the splenocyte proliferations stimulated by concanavalin A and LPS in a concentration-dependent manner. Moreover, in ovalbumin-immunized mice, intraperitoneally administration of *A. annua* ethanol extract at a single dose of 0.25, 0.5 and 1.0 mg significantly reduced the ovalbumin-specific serum lgG, lgG1 and lgG2b antibody levels and suppressed the splenocyte proliferation. Taken together, *A. annua* did showed immunoregulation activities, but it deserved more studies to be developed as immunmodulator.

3.6.2. Anti-adipogenic activities

Artemisinic acid was the firstly found component derived from *A. annua* proved to possess anti-adipogenic activities *in vitro* (Lee et al., 2012). It was reported that artemisinic acid could inhibit adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells through reducing the expression of CCAAT/enhancer binding protein (C/EBP)δ mediated by inhibiting Jun N-terminal kinase (JNK). With this revelation, the anti-adipogenic activities of *A. annua* extracts and *A. annua* essential oil were evaluated both *in vitro* and *in vivo* (Baek et al., 2015; Hwang et al., 2016; Song et al., 2017). When 3 T3-L1 cells were treated with *A. annua* leaves extract (25 and 100 μg/mL), adipocyte differentiation was markedly suppressed *via* inhibiting dexamethasone, 3-isobutyl-1-methylxanthine and insulin-induced Akt activation and the expression of adipogenic genes, including C/EBP α and peroximal proliferator-activated receptor-γ (PPARγ) (Song et al., 2017). Meanwhile, *A. annua* leaves extract also suppressed the expression of adipocyte fatty acid-binding protein 4 (FabP4), a known PPARγ-target gene. In high-fat diet (HFD)-induced obese rats, oral administration of *A. annua* leaves extract (150 mg/kg) significantly decreased HFD-induced weight gain, fat deposition, and adipose cell size, and alleviated serum total cholesterol (TC) and triglyceride (TG) levels.

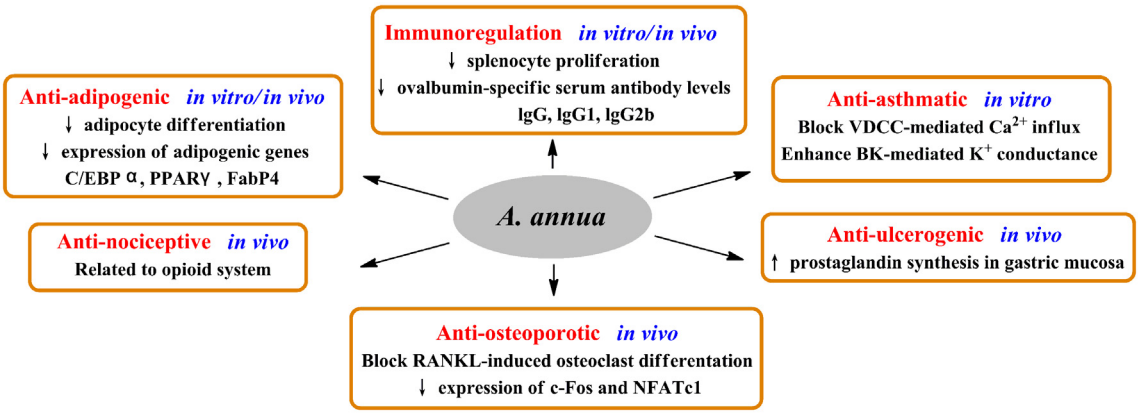

Fig. 3. Overview of the proposed modes of action of *A. annua* for its immunoregulation, anti-adipogenic, anti-ulcerogenic, anti-asthmatic, anti-nociceptive and anti-osteoporotic activities. ↑ indicates upregulation and ↓ indicates downregulation.

Similar results were obtained when 3 T3-L1 cells or HFD-induced obese mice were treated with *A. annua* water extract or *A. annua* essential oil (Baek et al., 2015; Hwang et al., 2016). All these results suggested that *A. annua* could be a promising therapeutic for preventing obesity and related metabolic disorders.

3.6.3. Anti-ulcerogenic activities

In indomethacin-induced ulcer rats, orally administration of *A. annua* crude ethanol extract at the dose of 500 mg/kg inhibited the ulcerative lesion index (ULI) by 53.8% (Dias, Foglio, Possenti, Nogueira, & de Carvalho, 2001). An enriched sesquiterpene lactone fraction (SLF) purified from *A. annua* crude ethanol extract showed similar effects (86.1% ULI inhibition for i.g. and 59.8% ULI inhibition for s.c.). Then, three different polarity fractions (non-polar, medium polarity and polar fraction) were prepared from SLF by column chromatography and their anti-ulcerogenic activities were evaluated. Non-polar, medium polarity and polar fraction treatment (500 mg/kg, i.g.) inhibited the ULI by 88.3%, 57.7% and 31.1%, respectively in indomethacin-induced ulcer rats. Pharmacological mechanism studies indicated that *A. annua* exhibited anti-ulcerogenic activities *via* increasing the prostaglandin level in gastric mucosa. Three sesquiterpene lactones were isolated from SLF, namely artemisinin, dihydro-epideoxyarteannuin B and dexyartemisinin (Foglio et al., 2002). Dihydro-epideoxyarteannuin B and dexyartemisinin showed anti-ulcerogenic activities in both indomethacin- and ethanol-induced ulcer rats. However, no cytoprotection effect was observed for artemisinin.

3.6.4. Anti-asthmatic activities

The anti-asthmatic activities of *A. annua* was investigated *in vitro* using tracheal rings (TRs) and acute isolated airway smooth muscle cells (ASMCs) of mice (J. Huang et al., 2017). Chloroform extract of *A. annua* significantly inhibited high K^+-induced contraction on mouse TRs in a dose-dependent manner ($IC_{50} = 0.316$ mg/mL). Meanwhile, chloroform extract of *A. annua* could also abolish ACh-induced contractions. The underlying mechanisms were explored using patch-clamp technique and ion channel blockers, indicating that blocking voltage-dependent Ca^{2+} channel-mediated Ca^{2+} influx played an important role, and enhancing Ca^{2+}-activated K^+-mediated K^+ conductance played a less important role in the anti-asthmatic activities of *A. annua*.

3.6.5. Anti-nociceptive activities

In Favero's study, an enriched sesquiterpene lactone fraction (Lac-FR) with 1.72% artemisinin and 0.31% deoxiartemisinin content was isolated from *A. annua* residue (the artemisinin had already been extracted) and investigated for its anti-nociceptive activities in various chemical-induced nociception in mice (Favero Fde et al., 2014). Intraperitoneally administration of Lac-FR (30, 100 and 300 mg/kg) significantly reduced the reaction time of mice in both phases of the formalin test, the sensitivity to mechanical allodynia stimulus, carrageenan-induced paw edema, acetic acid-induced abdominal constrictions. Also, Lac-FR was effective in tail flick model, indicating that opioid system was involved in its anti-nociceptive activity.

3.6.6. Anti-osteoporotic activities

In vivo anti-osteoporotic activities of *A. annua* and its active components were investigated in ovariectomized (OVX) mice (Lee et al., 2017). After the OVX mice were orally administered with *A. annua* ethanol extract (1 and 10 mg/kg), OVX-related changes in bone morphometric parameters, including decreased bone volume over total volume and trabecular number, and increased trabecular separation were markedly suppressed. Meanwhile, the levels of osteoporosis-related serum markers were significantly reduced, and the increase in the serum levels of proinflammatory cytokines (TNF-α and IL-1β) was inhibited when OVX mice were treated with *A. annua* ethanol extract. Similar results were obtained when OVX mice were treated with artemisinin (10 and 20 mg/kg) or arteannuin B (20 mg/kg), which were the major components of *A. annua*. 17β-estradiol was used as a positive control and the anti-osteoporotic activities of *A. annua*, artemisinin and arteannuin B were comparable to those of 17β-estradiol. Further studies revealed that *A. annua*, artemisinin and arteannuin B exhibited anti-osteoporotic activities by blocking receptor activator of nuclear factor kappa-B ligand (RANKL)-induced osteoclast differentiation *via* reducing the expression of the two transcription factors, c-Fos and NFATc1.

4. Novel components isolated from *A. annua* and their biological activities

A. annua had been one of the most widely investigated herbs since the isolation of artemisinin in 1972. During the past few decades, phytochemical investigations have demonstrated that sesquiterpenoids, flavonoids, coumarins, triterpenoids and phenolics were the main components existing in *A. annua* (Bhakuni, Jain, Sharma, & Kumar, 2001). Even though over 600 components were isolated and identified, investigators were still doing their best to fully elucidate the phytochemical profiles of *A. annua* (Brown, 2010). In this section, novel components isolated during the past twenty years and their biological activities were summarized (Chu, Wang, Chen, & Hou, 2014; Li et al., 2015; Li et al., 2019; Qin et al., 2018; Zhai, Supaibulwatana, & Zhong, 2010). As showed in Fig. 4, eight sesquiterpenoids (components **1**, **2**, **3**, **4**, **6**, **7**, **12** and **13**), two coumarins (components **9** and **10**), two

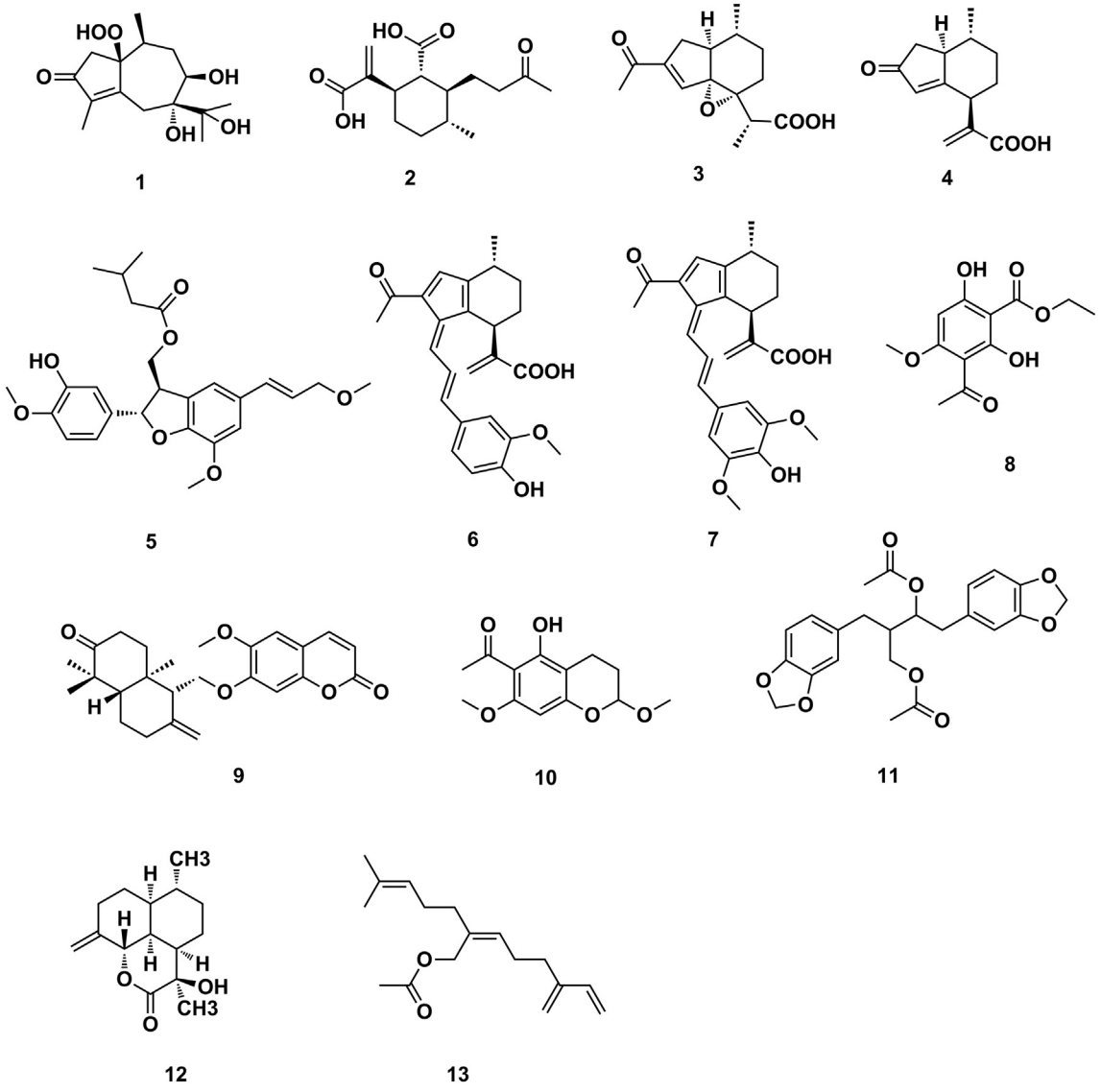

Fig. 4. Novel components isolated from *A. annua* in the last twenty years (2000—2020).

lignans (components **5** and **11**), and one phloroglucinol derivative (component **8**) were isolated from *A. annua* in the last twenty years.

Compounds **10**, **8** and **11** exhibited anti-fungal activities against *Fusarium oxysporum*, *Fusarium solani* and *Cylindrocarpon destrutans* (Li et al., 2019). Compound **10** inhibited all these three fungi with MIC values of 18.75, 18.75 and 25.00 μg/mL, respectively. Compounds **8** and **11** showed anti-fungal activities against *Cylindrocarpon destrutans*. Anti-inflammatory activities of compounds **6** and **7** were evaluated *in vitro* (Qin et al., 2018). They significantly inhibited the NO production in LPS-activated RAW 264.7 cell lines with IC_{50} of 4.5 and 2.9 μM (hydrocortisone as positive control, IC_{50} = 48.7 μM). Compound **13** was reported to possess anti-cancer activity (Zhai et al., 2010). MTT assay revealed that compound **13** showed cytotoxic activities against various human cancer cell lines, including HP8910 (ovary), 95-D (lung), QGY (liver) and HeLa (cervix), with IC_{50} values ranges from 52.44 to 73.3 μM. Further studies showed that compound **13** could induce the apoptosis of lung 95-D tumor cells *via* mitochondria dependent pathway. The promising biological activities of these novel components combined with their unique architectures provide valuable inspiration for drug discovery.

Besides the low-molecular components, several high-molecular components isolated from *A. annua* such as polysaccharides have also been reported (Chen et al., 2013; Huo, Lu, Xia, & Chen, 2020; Yan et al., 2019). In the study conducted by Yan et al., a polysaccharide was isolated and identified from *A. annua* (Yan et al., 2019). *In vitro* study proved that this polysaccharide was able to inhibit the growth of HepG2 cells *via* p65-dependent mitochondrial signaling pathway. In another study, three polysaccharides (AAP01–1, AAP01–2 and AAP01–3) were isolated and investigated for their anti-complement activities (Huo et al., 2020). AAP01–2 showed potent anti-complement activity (CH_{50} = 0.36 mg/mL, AP_{50} = 0.547 mg/mL), while AAP01–3 showed slightly anti-complement activity and AAP01–1 was inactive.

5. Current developments and limitations of *A. annua*

As described in this review, *A. annua* has been proved to possess a variety of pharmacological activities (Fig. 5). Compared with its recommended therapeutic usages recorded in ancient Chinese medical textbooks, there are still several traditional usages are not estimated by modern pharmacological researches, including wounds, dysentery, haemorrhoids, rhinopolyp, tuberculosis, et al. Further investigations are still needed to fully reveal the potential clinical application of *A. annua* and the following aspects are worth addressing.

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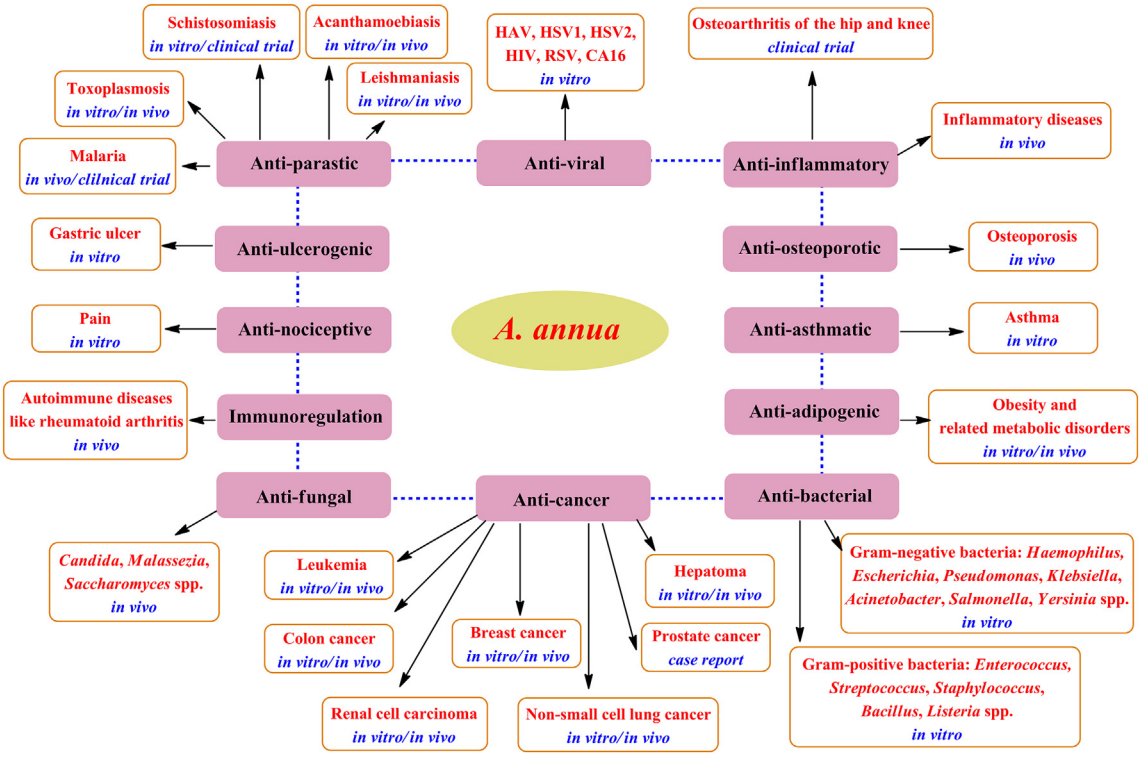

Fig. 5. Biological activities of *A. annua* and the potential clinical applications.

Firstly, it is generally known that herbal drug formulation preparation techniques affect the therapeutic outcome. In Ge Hong's Handbook of Prescriptions for Emergencies, "soaking a handful of plant in two liters of water, then wringing it out and ingesting the juice in its entirety" was recommended for the treatment of malaria. This may create an emulsion of the water with the essential oils, flavonoids, and quinic acids. Currently, in Chinese Pharmacopeia, *A. annua* is recommended to be prepared as tea infusion (dried *A. annua* leaves immersed in hot water). However, in the reported studies summarized in this review, different *A. annua* products were involved, including *A. annua* tea infusion, solvent extracts, fractions, essential oils, polysaccharids, polyphenols and active components. In this case, results obtained from different research groups were hard to replicate and sometime were even contradictory. Additionally, chemical profiles of *A. annua* could be influenced by the harvesting season, the geographic location, fertilizer, the choice and stage of drying conditions, extraction method et al., making it more difficult to assess the results gained from different groups. Thus, clarification of the chemical profiles and development of standard operating procedures for the *A. annua* products will be crucial in further research.

Secondly, pharmacological activity research of *A. annua* is only in its infancy except for the anti-malarial. Even though several clinical trials were involved, most of the biological activity investigations still remained at preclinical studies (Fig. 5). Studies could reveal the mechanisms of *A. annua* on the molecular biological levels are so woefully insufficient. For example, the anti-bacterial activities of *A. annua* essential oil were widely evaluated; however, most of the investigations were at the level of *in vitro* studies. Thus, whether *A. annua* essential oil was effective for the treatment of bacterial infection still need to be further studied and established. Similar situations occurred with the anti-viral activities of *A. annua*. Meanwhile, although *A. annua* had been proved to be safe in clinical application for the treatment of malaria, chronic toxicological studies for long-term use of *A. annua* in other diseases were still needed.

Thirdly, it was claimed that the anti-cancer action of *A. annua* was superior to the single purified component, indicating that synergistic effects exist (van der Kooy & Sullivan, 2013). On one hand, the biological effect could be a synergism of all the molecules contained in *A. annua*. On the other hand, it is also possible that the biological activities of the main components could be modulated by other minor components, and the activities of the main components are also distinguished. Until now, only flavonoids were reported to possess synergistic effects with artemisinin against malaria and cancer *via* its immunoregulation activity and inhibitory activity of CYP450 enzymes (Ferreira, Luthria, Sasaki, & Heyerick, 2010). It is worthwhile to look into the great potential synergistic effects in *A. annua*.

Fourthly, it was believed that pharmacological activities of *A. annua* were attributed to its diverse chemical components. There was no doubt that artemisinin was the most successful drug derived from *A. annua* which helped to save millions of lives. Other components like artemisinic acid, casticin, chrysosplenol D and β-caryophyllene had also been widely studied. Artemisinic acid was reported to possess anti-adipogenic activity. Casticin and chrysosplenol D exhibited anti-inflammatory and anti-cancer activities. β-caryophyllene showed significant leishmanicidal effect. Even though clinical study was still needed to further prove the effectiveness of these components, they were still merit exploration as potential therapeutics.

In addition to the numerous pharmacological researches, *A. annua* had also been widely investigated in other aspects. Firstly, artemisinin, the main active component of *A. annua*, and its derivatives artesunate, artemether, arteether, dihydroartemisinin and artemisone were extensively investigated. As first-line drugs for malaria treatment, artemisinin and its derivatives were proved to be efficient and low-toxicity. Recent studies demonstrated that they also exhibited beneficial effects in cancer, viral diseases, immune diseases, parasitic infections, which were covered by considerable amount of excellent reviews (Crespo-Ortiz & Wei, 2012; Efferth et al., 2008; Frohlich, Capci Karagoz, Reiter, & Tsogoeva, 2016; Lam, Long, Su, & Lu, 2018; Lam, Long, Wong, Griffin, & Doery, 2019; Liu, Cao, Huang, Zhao, & Shen, 2019; Loo, Lam, Yu, Su, & Lu, 2017; Mu & Wang, 2018; Saeed ur et al., 2019; Slezakova & Ruda-Kucerova, 2017; Wong et al., 2017). Secondly, *A. annua* is the

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only commercial source of artemisinin and naturally artemisinin is produced in small quantities. Thus, there are continuous efforts to increase artemisinin supply such as transgenic approach to enhance the artemisinin yield in plants, semi-synthesis of artemisinin *via* artemisinic acid in yeast and chemical synthesis (Ikram & Simonsen, 2017; Lv, Zhang, & Tang, 2017; Shen, Yan, Fu, & Tang, 2016; Tang, Shen, Yan, & Fu, 2014; Xiao, Tan, & Zhang, 2016). Thirdly, resistance to ACTs has recently been reported in Southeast Asia and understanding artemisinin resistance is another hot research topic. More and more researches were carried out to elucidate the working mechanism of artemisinin resistance at molecular level and provide potential ways to overcome resistance (Conrad & Rosenthal, 2019; Suresh & Haldar, 2018; Talman, Clain, Duval, Menard, & Ariey, 2019; Tilley, Straimer, Gnadig, Ralph, & Fidock, 2016; Wang, Xu, Lun, & Meshnick, 2017).

6. Summary

In summary, extensive *in vitro* and *in vivo* data have revealed that *A. annua* possess excellent anti-malarial effects and multiple other biological activities, including anti-parastic, anti-viral, anti-fungal, anti-bacterial, anti-inflammatory, anti-cancer, anti-adipogenic, anti-osteoporotic, anti-asthmatic, anti-ulcerogenic, anti-nociceptive and immunoregulation, supporting the promising therapeutic application of *A. annua* in various human diseases. For the next decade, more clinical indications would be found with more pharmacological mechanism of *A. annua* being revealed. We hope this review could provide a scientific basis for further investigations to assess mechanism underlying the effects and clinical applications of *A. annua*.

Declaration of Competing Interest

The authors have no conflict of interest.

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