Chapter 14
Reverse Pharmacology and Drug Discovery: *Artemisia annua* and Its Anti-HIV Activity

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14.1 Introduction

14.1.1 Drug Discovery and Reverse Pharmacology

There are various ways in which new drugs can be developed. One approach is *in silico* drug design based on our existing knowledge of the biology of a specific disease and the specific target site binding chemistry. Based on this knowledge, a range of molecules will be designed and synthesised after which they will be tested in in vitro bioassays for activity and toxicity. The best candidates, called lead compounds, will then be "fine-tuned" by chemical derivatisation in order to improve their activity and/or to reduce their toxicity. Lead compounds are then tested in various animal models before entering clinical trials in people. Another approach is to screen a large number of biological samples (plants, bacteria and fungi) for activity against a specific disease. Any active extract, consisting of many compounds, will be fractionated by chromatographic techniques, and each fraction will be tested for in vitro activity. Active fractions will again be fractionated until the active compound is identified. This process, also called bioguided fractionation, can go through a number of fractionation cycles before the active compound is identified. The active compound will be chemically derivatised in order to improve its properties before in vivo animal studies will be conducted. Based on these test results, the most promising lead compounds will then be tested in clinical trials in people. There are however a number of shortcomings with both approaches. It is expensive, time consuming, makes use of in vitro bioassays and it suffers from a very low success rate. Due to these shortcomings, it is currently estimated that the development of one new drug costs around $1–1.5 billion, simply because so many lead compounds fail during clinical trials. Keeping these high costs in mind, one would think that all

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registered drugs are effective and importantly non-toxic. Unfortunately, this is not the case, as there are a number of drugs currently on the market that are causing severe side effects and whose efficacy should be questioned. This holds true particularly for cancer chemotherapeutics. It was estimated that cancer chemotherapy improves the average 5-year survival rate of patients (for all cancer types) by only 2 % (Morgan et al. 2004). Another relatively unknown fact is that each year, 200,000 people die in the EU due to adverse drug reactions (all types of drugs), highlighting the severe shortcomings of the drug development and drug licensing pipelines (Archibald and Coleman 2012). To put this into perspective, there are a large number of drugs that work perfectly well and are safe to use, but we have to concede that our approach to drug discovery and our overall approach to health care suffers from some major problems.

There are alternative approaches in which these problems can partly be sidestepped. One such approach can be called reverse pharmacology. This relatively simple approach identifies traditional medicines (e.g. predominantly medicinal plants) that are known to be non-toxic in vivo. This is an extremely important aspect as most lead compounds identified during the normal drug discovery processes fail during clinical trials due to in vivo toxicity. If we identify medicinal plants that have been used and are still being used by thousands of people without any reports of toxicity, we have practically sidestepped this major problem. The second step in reverse pharmacology is to work our way back to the active compound(s) with the use of in vitro bioassays. We therefore start with the in vivo uncontrolled clinical trial followed by the in vitro identification of the active compound(s). One notable difference with the normal drug discovery process is that this system does not necessarily need in vivo animal studies to be conducted (however, the drug registration authority demands animal studies to be conducted before registration). The reverse pharmacology approach is however not without its shortcomings either. The main problem here is that in vitro bioassays are being used, leading to the same set off problems experienced with the normal drug discovery pipeline at this stage of the process. Some of the main problems with in vitro bioassays are as follows:

- They exclude the influence of human metabolism on the active compounds and will therefore not detect the so-called prodrugs. Prodrugs are compounds that are inactive in their natural form but are chemically altered during normal metabolism to become active.
- Bioassays are designed to identify compounds with a specific mechanism of action. It can for instance consist of a single enzyme (e.g. HIV integrase) or a specific life cycle stage of a parasite. Any active compound that does not bind to this enzyme or does not act against a specific life cycle stage might therefore be deemed inactive—A so-called false negative.
- Bioassays do not test for important pharmacokinetic and dynamic aspects such as bioavailability. If the compound is not bioavailable in vivo, then the in vitro activity does not matter much (bioavailability can however be improved by encapsulation techniques).

- Bioassays normally make use of "foreign" organic chemicals or media that can react and thereby deactivate active compounds, leading to a large number of false negatives. Common compounds found in many plants can also react with these bioassay media constituents, making them active, leading to a large number of false positives.

The main issue is however the problem of false positives and false negatives. The false positives are compounds that are deemed "active" in a specific bioassay after which a large amount of time and effort will be spent on their identification. Fortunately, false positives eventually always reveal themselves but, unfortunately, at a cost of time and funds. False negatives are a far more serious problem in that they do not react with a specific enzyme in the bioassay used and are therefore deemed inactive. They may even degrade in the "foreign" media used in the bioassay. The main problem here is that false negatives never reveal itself. For example, if it is published that medicinal plant X show no activity against disease X, very few people will go and reinvestigate, if any. Unfortunately, this problem is also important when using in vitro bioassays to test for the toxicity of lead compounds or active extracts. Here, again we find the occurrence of false positives and negatives. False positives are again compounds reacting with specific cells, leading to cell death and the conclusion is made that this compound or extract is toxic. A good example of this was that we recently tested English tea (*Camellia sinensis*) as negative control on a specific cell line. Due to toxicity in that specific cell line, our experiments could not continue, and we could not use normal tea as a negative control (unpublished results). Many millions of people are drinking tea every day without any short- or long-term toxicity reported thus far. To put this into perspective, if this was a medicinal plant, we would have concluded that it is far too toxic and we would not have continued our investigations. A false negative in toxicity bioassays speaks for itself. There are countless examples of lead compounds that were deemed safe after in vitro and even in vivo animal studies that turned out to be toxic in people.

There are many problems associated with in vitro bioassays, and it will remain a problem for the normal drug discovery pipeline as well as for the reverse pharmacology approach. The only advice is to interpret in vitro results very carefully.

14.1.2 Reverse Pharmacology: Economic and Political Considerations

These two approaches to drug discovery that we can call the "normal" and "reverse" approaches are, unfortunately, at the moment exclusive. The main reason for this is not only scientific but economic and political, and that is why this aspect should shortly be discussed here. We live in a segregated world with on the one side the "rich" Western countries with their own unsustainable complex

economic system (capitalism) and, on the other side, the "poorer" non-Western countries with their more sustainable simpler economic systems. In Western countries, scientific research should lead to the creation of financial profits in the short term in order to sustain their complex economic system. In practice, this means that funding for research into (new) medicines will be made available only if research will lead to the development of a new drug that can be sold for a profit in order to create or sustain a company and keep people employed. In short, to keep the economy growing, profits need to be made. As such, there is nothing wrong with this approach, but it does however focus on the creation of wealth and employment on the short term, and not on the health of people. One advantage of this economic system on a scientific level is that these countries have extremely well-equipped laboratories to perform in-depth scientific research into new medicines, but as long as potential profits take centre stage. Because of this, research into medicinal plants has basically been stopped for the simple reason that a medicinal plant cannot be produced and sold in order to create wealth—it is just too inexpensive and will therefore not be able to financially sustain anyone or any company. Identifying a new lead compound from a medicinal plant will be supported to some degree, but only if the possibility of patenting and eventual profits exist. The harsh reality is that if a medicinal plant works or not (the science behind it) does not really matter at all—it just does not fit into the economic model. In non-Western countries, the focus is still primarily on the health of people and not on short-term direct profits. Here, medicinal plants have always played a role and will continue to do so as long as the main objective is the health of their citizens and not direct short-term profits. That people sell medicinal plants for a profit in these countries does of course take place, but this is not on a comparable scale to the cost of drugs in Western countries.

Is there any possibility to reconcile these two worlds? Can and should research into medicinal plants take place in Western countries in order to use it as such? The way we see it is that we do not have a choice anymore. The health care systems in many Western countries are on the verge of collapse, and it appears that the capitalistic model and health care are not a good mix after all. A fast growing lack of trust and the extremely high costs involved are probably the main reasons. It has become too expensive, proven by the fact that many people in Western countries are already excluded from primary health care, simply because they cannot afford it. We have to find a way to reduce the cost of health care in Western countries. Expensive medicine is of course only one part of the overall cost of the health care system, but it is a good starting point. What needs to be done is to study the health care systems in non-Western countries and to apply our vast resources in Western countries into studying specifically the types of medicine that they use in order to provide and create an evidence-based, quality-controlled, inexpensive and effective medicine. We have to be clear that not all of the traditional medicines used in non-Western countries will work; there will be many failures, but there will (or might) also be big successes. One medicinal plant that should be studied in detail is *Artemisia annua* L. (Asteraceae) (Fig. 14.1). This medicinal plant has the potential to satisfy the current needs of both worlds but also has the potential to

Fig. 14.1 In the foreground, the *Artemisia annua* plant and in the background the users of this plant as a tea infusion for the treatment of various diseases (Photo with permission from Anamed)

bring these two worlds closer together. It can yield single compounds that can be further developed into a drug—which it already did. It can also be used in the form of a medicinal plant as an inexpensive, effective treatment for life-threatening diseases in non-Western countries—which it already did. Our sincere hope is that this plant will one day even be used in Western countries as soon as the scientific evidence for its efficacy is provided. All we need to do is some in-depth scientific research, which at the moment is unfortunately not being supported in Western countries due to the aforementioned economic reasons. To address this, we have recently published a review paper dealing with the scientific complexities we face in studying this plant and recommended the logical next steps that should be taken (van der Kooy and Sullivan 2013). This chapter will therefore describe another important aspect of *A. annua* (Fig. 14.1) and the discovery of the remarkable anti-HIV activity of this plant. We will give a short introduction on the history of *A. annua*, the biological activities associated with this plant, the reported antiviral activities and more specifically the anti-HIV activities of all compounds identified in the traditionally prepared *A. annua* formulation. The potential impact of this discovery and the need to continue with this research will also be discussed.

14.1.3 Background of Artemisia annua and Artemisinin

A. annua has been used for hundreds of years to treat various ailments, specifically those related to the treatment of intermittent fevers. This traditional use has been linked to malarial infections caused by the *Plasmodium falciparum* parasite. From *A. annua,* one active compound, a sesquiterpene lactone endoperoxide called artemisinin has been identified and is currently being used, as artemisinin derivatives, as a first-line treatment for malaria (De Ridder et al. 2008). The traditional use of *A. annua* in the form of a tea infusion prepared from the dried leaves

continues to be used in China and due to its apparent effectiveness has rapidly spread across the globe in recent times. Currently, it is being used all over the world as an inexpensive treatment for malaria, as opposed to the WHO-recommended artemisinin derivatives. The traditional use of *A. annua* has caused a large amount of controversy, and it is feared that resistance against artemisinin might develop because of the traditional use of the plant. There is however an opposing view that states *A. annua* has been used for hundreds of years without resistance developing and only now since a single compound from this plant is being used, resistance has developed. The use of *A. annua* can however have important implications specifically for antimalarial treatments in developing countries, where the high cost of commercial drugs makes it practically inaccessible to many. It can also have important implications for Western countries where the general high cost of health care has already excluded many from receiving adequate treatment for disease. The common view on the use of *A. annua* supported by the WHO is relatively simplistic. In order to reach the prescribed doses of the main active compound artemisinin, they have concluded that artemisinin combination therapies (ACTs) are the only way to approach the treatment of malaria (Mueller et al. 2004; WHO 2012). This clash between Western-based drugs (e.g. artemisinin) and traditional medicines (medicinal plants e.g. *A. annua*) has culminated in the publication of a Position Statement by the WHO on the use of *A. annua* for the treatment of malaria. The main recommendation in this statement was not to use *A. annua* in any form including a tea infusion due to the low natural abundance of artemisinin in *A. annua* (Liu et al. 2006 and WHO, 2012). For a full discussion on the traditional use of *A. annua,* Hsu (2006) should be consulted, and for a full discussion on the controversy surrounding the use of *A. annua* versus the WHO-recommended use of artemisinin derivatives, van der Kooy and Sullivan (2013) should be consulted.

There are however some problems relating to the use of artemisinin. The main problem is that it is a very fast-acting drug with a very short half-life. It therefore needs to be combined with a slower-acting drug in order to assure the effective elimination of all the parasitaemia. Another problem is the assurance of an adequate supply of artemisinin. Due to its complex chemical structure, the synthesis and/or semi-synthesis remains to be difficult, and therefore the main source of artemisinin remains to be isolation and purification from the *A. annua* plant. Due to environmental and economic influences, the annual production of *A. annua* suffers from a boom and bust cycle with overproduction and low prices the one year to underproduction and high prices the next year. The cost of this Western-style treatment is also far too high for where it is used—predominantly in non-Western countries. In order to stabilise the global production of *A. annua* and the price of artemisinin, various approaches have been taken. To increase the yield of artemisinin in *A. annua,* classical plant breeding techniques have been employed (Bartlet 2010); the isolation and purification techniques are continuously being improved (Liu et al. 2011a), and the chemical synthesis of artemisinin has been successful to some degree (Dietrich et al. 2009). It is of note that all of this research has taken place in Western countries and that the focus is on the single compound artemisinin. Another novel approach would be to identify a second

active pharmaceutical ingredient (API) in order to reduce the overall production cost of artemisinin (Lubbe et al. 2012). In order to do this, the traditional and current use of *A. annua* for diseases other than malaria was investigated.

14.2 Anti-HIV Activity of *Artemisia annua*

14.2.1 The Antiviral Activity of Artemisia annua and Artemisinin

A. annua is currently mainly being used for the treatment of malaria but also for the treatment of skin and digestion ailments, HIV-AIDS, bronchitis, cancer and haemorrhoids. These alternative uses for this medicinal plant is recommended by NGOs such as IFBV-BELHERB and Anamed (2012). This section will however only deal with the antiviral activities associated with *Artemisia* spp., and artemisinin.

Reports of other *Artemisia* species showing anti-HIV activity are limited to *A. caruifolia* and *A. capillaris.* Ma et al. (2001) identified four compounds from methanol extracts of *A. caruifolia,* namely *N*1, *N*5, *N*10-tri-*p*-coumaroylspermidine derivatives which showed around 70 % inhibition of HIV-1 protease at a concentration of 100 μg/mL. The three dicaffeoylquinic acid derivatives also isolated during this study did not show any appreciable activity against HIV-1 protease. However, Cos et al. (2008) reported that 3,5-dicaffeoylquinic acid did indeed show good activity against HIV integrase, although controversy remains around its potency and activity in vivo. In a metabolomic investigation of *A. annua* and *A. afra*, coumaroylspermidine derivatives were not detected in either species tested (Liu et al. 2010). No other reports could be found that these compounds have been identified in *A. annua*. Wu et al. (2001) concluded that the active anti-HIV components present in *A. capilaris* were the flavonoids isorhamnetin and arcapillin, as well as the coumarin derivative aesculetin.

A. annua has been shown to possess a wide variety of antiviral effects, notably against herpes simplex virus, Type 1 (HSV1). Karamoddini (2011) conducted in vitro tests on methanolic extracts of homogenised plant material of various *Artemisia* species, where *A. annua* showed the highest antiherpetic activity. *A. annua* was also tested with the common antiviral drug acyclovir, where it showed higher anti-HSV1 activity than the commercial drug (Karamoddini 2011). It is possible that the noted antiherpetic effects could be caused by artemisinin; however, synergism or other active compounds has yet to be tested in relation to HSV1. *A. annua* has been shown to be active against the severe acute respiratory syndrome-associated coronavirus, a highly deadly and contagious disease (Li et al. 2005). Li et al. (2005) tested ethanolic extracts of *A. annua* and three other plant species against the virus, determining *A. annua* to have an EC_{50} of 34.5 ± 2.6 μg/mL. Abid Ali Khan et al. (1991) studied the antiviral activity of *A. annua* against the

tobamoviruses, where plant samples were extracted with *n*-hexane and evaluated against tobamovirus virus cultures, with 75 % inhibition noted. Abid Ali Khan et al. (1991) then fractionated the extract, collecting the active fraction and determined the active compound/s to be sterols. The outcome of this study is important to note as artemisinin was not identified as the virus inhibitor.

The pure compound artemisinin also showed significant activity towards the bovine viral diarrhoea virus (BVDV) (Romero et al. 2006). BVDV is classed as a pestivirus, under the *flaviviridae* family, and in this study, bovine epithelial cells were infected with the BVDV virus and treated with artemisinin. It was found that artemisinin is an inhibitor of *flaviviridae* viruses, with a possible application to the treatment of the hepatitis C virus (HCV). HCV is very similar to HIV in that they are both RNA viruses; however, HIV is a retrovirus and integrates itself into the hosts DNA. Paeshuyse et al. (2006) confirmed this by testing artemisinin against HCV and found it to be active at levels of 78 ± 21 μM. This activity was potentiated by hemin at concentrations that had no effect on the host cells, indicating a pronounced synergistic antiviral activity when artemisinin and hemin were combined. A further understanding of how *A. annua* extracts, and not just artemisinin, interact and inhibit viruses could enable future investigations to discover and develop practical and inexpensive treatments for viruses of a similar nature, like that of HIV. Romero et al. (2005) studied the activity of artemisinin against hepatitis B virus (HBV), noting IC_{50} values of 55 and >100 μM for the inhibition of hepatitis B envelope protein surface antigen and HBV-DNA release, respectively. Interestingly, artemisinin proved to be a more effective antiviral than its commonly used derivative, artesunate, with IC_{50} values of 2.3 and 0.5 μM, respectively.

14.2.2 The Anti-HIV Activity of Artemisia annua and Artemisinin

In vivo activity: A 2011 survey completed by Willcox et al. (2011) in Kenya and Uganda reported that 51 % ($n = 20$) of respondents were using *A. annua* infusions for diseases other than malaria, where 28 % ($n = 11$) were using the infusions for the treatment of HIV. Furthermore, a survey completed by Noumi and Manga (2011) in Cameroon also noted that 13.8 % of respondents were using *A. annua* in relation to HIV/AIDS. Abu-Raddad et al. (2006) hypothesised that the spread of the malaria parasite may be enabling the spread of the HIV virus throughout Africa, due to tainted blood used for transfusions. This hypothesis gives reason to the further study of current malaria treatments in relation to HIV, like that of *A. annua* and other commonly used medicinal plants.

In vitro activity: Information regarding the in vitro anti-HIV activity of *A. annua* and artemisinin is rather limited. Chang and Woo (2003) tested the methanolic extracts of 80 commonly used Korean medicinal plants against HIV in relation to virus—cell fusion inhibition. They used the syncytium inhibition assay,

which is based on the interaction between the HIV-1 envelope and the cellular membrane protein CD4 on T-lymphocytes. Some inhibition was seen at the concentration tested (15.8 % ± 5.9), even though it was relatively low compared to some of the other 80 plants in the test series and therefore no other solvent extracts or fractions of *A. annua* were tested during this study. In reality, organic extracts are not used, but rather a tea infusion is brewed and administered. Various patents exist that cover the broad biological activity including the antiviral activity of *A. annua* in combination with other medicinal herbs (Zhang and Zhang 2010; Chen 2010; Nagaura 2009; Xue 2008a, b; Zhang 2003; and Chen 2007).

The first report on artemisinin derivatives being used as anti-HIV agents was published by Jung and Schinazi (1994) who embarked on a study of artemisinin trioxane derivatives. They concluded that anti-HIV activity was common in artemisinin trioxane derivatives and, given their results, further evaluation was needed for a potential anti-HIV treatment to be produced. Lubbe et al. (2012) investigated the traditional infusion of both *A. annua* and *A. afra*, compared to pure artemisinin, in relation to anti-HIV activity, noting that both infusions exhibited potent inhibition with *A. annua* having an IC_{50} of 2.0 μg/mL, whereas pure artemisinin showed no inhibition even at 25 μg/mL. *Artemisia afra* which does not contain artemisinin showed a similar level of activity against HIV, indicating that the active compounds are probably not artemisinin. Given the surveys completed by Willcox et al. (2011) and Noumi and Manga (2011), a clear relationship between plant and disorder can be seen. The implementation of reverse pharmacology in cases like this would enable researchers to possibly develop effective treatments for various viruses (specifically HIV) and diseases, given their socio-historical validation.

14.2.3 The Discovery of the Anti-HIV Activity of the Artemisia annua Tea Infusion

Keeping in mind the economic and political aspects surrounding the discovery and development of new drugs, we embarked on a non-funded project in order to study the possibility of reducing the price and the accompanying price fluctuations of artemisinin by identifying a second API in *A. annua*. A reverse pharmacology approach was taken, which means that we had to find evidence that *A. annua* is being used by thousands of people without any reports of toxicity. In many parts of the world, specifically in non-Western countries, people rely on traditional medicines as primary source for their health care needs (Farnsworth 1985). For a relatively new disease such as HIV, the identification of medicinal plants that work against these new diseases will therefore still undergo the process of trial and error. Nevertheless, the small-scale surveys conducted by Wilcox et al. (2011) and Noumi and Manga (2011) revealed that HIV-infected patients had started to use *A. annua* infusions for the treatment of HIV although the original treatment was intended for malaria. In both these surveys, the patients prepared a tea infusion

from *A. annua* either alone or with other plant species. Feedback from the non-governmental organisation Anamed also claimed that the herb is often used by HIV patients. This information is important in that we know that *A. annua* is being used by thousands of people for the treatment of malaria without any toxicity reported thus far. In effect, a large uncontrolled clinical trial has been conducted exactly where it should take place, where the highest burden of HIV occurs. This has given us two important outcomes: The *A. annua* tea infusion appears to be non-toxic and exhibits claimed in vivo anti-HIV activity. This is the first important step as toxicity is the biggest problem experienced in the drug discovery process. The next step in reverse pharmacology is to provide in vitro evidence that the traditional tea infusion does indeed have activity against HIV.

Two questions needed to be answered. Does the *A. annua* tea infusion exhibit any in vitro anti-HIV activity? And if so, is this activity caused by artemisinin? In order to test if artemisinin was responsible for the reported in vivo anti-HIV activity, we therefore had to include a chemically similar plant which does not contain any artemisinin. Previous work conducted on *Artemisia afra* Jacq. ex Wild. (Asteraceae) revealed it to be chemically similar to *A. annua* but, importantly, it does not contain artemisinin (Liu et al. 2010; van der Kooy et al. 2008). The inclusion of *A. afra* was done in order to determine whether artemisinin was responsible for any observed anti-HIV activity, or whether a combination of artemisinin and other components can explain any observed activity (synergism). Furthermore, we could not find any literature reports that *A. afra* was tested against HIV (Liu et al. 2009) except one report where HIV-infected patients were given *A. afra* together with standard HIV treatments in order to boost their immune systems (Mulholland and Drewes, 2004).

We prepared tea infusions from nine *A. annua* and one *A. afra* sample according to van der Kooy and Verpoorte (2011) and tested them against HIV according to Lubbe et al. (2012). The plant material was collected from different parts of the world and in different years. The results indicated that the *A. annua* tea infusions had IC_{50} values of between 0.6 and 3.7 μg/mL and for the *A. afra* infusion an IC_{50} value of 1 μg/mL (values corrected for variation in the positive control—Lubbe et al. 2012) (Table 14.1). These results by themselves are quite remarkable in that Cos et al. (2008) reported that any single compound with an activity of below 25 μg/mL should be considered to have significant antiviral activity. We know that the tea infusion prepared from *A. annua* consists of many compounds (Mouton et al. 2013), making this chemically complex "extract" highly active against HIV. Furthermore, the activities of all the samples appeared to be relatively similar, indicating that the concentration of the active compound in the samples is probably very similar. Furthermore, the active compound appears to be stable for a number of years as the oldest plant sample tested was about 10 years old. These results answered our first question in that the *A. annua* tea infusion does indeed exhibit good in vitro activity against HIV. We have to be careful in interpreting these results, and we have to keep in mind that in vitro bioassays suffer from some severe shortcomings as was discussed in the

Table 14.1 The *Artemisia annua* and *Artemisia afra* samples tested against HIV.

Sample	Country of cultivation	Harvest period	Plant parts	Artemisinin content	Corrected IC_{50} low (μg/mL)
Artemisia annua					
1	South Africa	1999	Leaves/flowers	0.36	1.0
2	South Africa	2002	Leaves/flowers	0.30	2.3
3	Tanzania	2005	Leaves	0.49	1.4
4	South Africa	2006	Leaves	0.74	1.2
5	Tanzania	2006	Leaves	0.46	1.3
6	Cameroon	2007	Leaves	0.56	2.6
7	Germany	2007	Leaves	0.58	4.5
8	Mozambique	2007	Leaves	0.40	0.6
9	Germany	2009	Leaves	0.80	3.7
Artemisia afra					
10	South Africa	>2008	Leaves	nd	1.0

nd = not detected

The country of cultivation, year of harvest, plant parts used and the artemisinin content of all the samples are given. The IC_{50} value against HIV has been corrected to compensate for the variation experienced with the positive control used in this bioassay according to Lubbe et al. (2012)

introduction of this chapter. The possibility exists that this result can be nothing more than a false positive. Unfortunately, only time will tell.

Another possible explanation for the anti-HIV activity is synergism between artemisinin and other compounds in the extract. With the inclusion of *A. afra* (not containing artemisinin) and the observed activity of this sample, it appears that artemisinin had a very limited role to play, if any. We have shown (van der Kooy et al. 2008 and Liu et al. 2010) that these two species are chemically closely related with the major exception that artemisinin has not yet been detected in any *A. afra* specimen. This was also one of the first reports that *A. afra* possesses significant in vitro anti-HIV activity (Lubbe et al. 2012) and provides scientific evidence to the reports of Mulholland and Drewes (2004) that patients given *A. afra* in combination with standard HIV treatment reported improvement of symptoms compared to patients taking only standard HIV treatments. *Artemisia afra* might therefore not only boost the immune system but may also have a direct activity against HIV.

The first step in the reverse pharmacology approach is relatively simple. We have identified a medicinal plant that has been used by thousands of people all around the world without any reports of toxicity. The second step is somewhat more difficult in that we now have to use in vitro bioassays in order to identify which compounds are responsible for the activity. As it stands, we do not yet know which compound(s) in *A. annua* or *A. afra* is responsible for its observed in vitro anti-HIV activity. Due to the reasons discussed in the introduction, research into the chemistry of the *A. annua* tea infusion will not be supported in any Western country. It is therefore interesting to note that very little chemical work has been done on the traditional tea infusion prepared from this plant. Due to this limited data, we can only report on what is currently known on the few identified compounds in the *A. annua* tea infusion regarding their possible anti-HIV activity.

14.3 The Chemistry of *Artemisia annua* Tea Infusions

There are a limited amount of chemical studies that have been performed on the *A. annua* tea infusion. Most of these studies have also focussed only on the extraction and quantification of artemisinin without conducting a complete chemical profile on the *A. annua* formulations.

Based on this limited data, the main conclusion was drawn that the tea infusion is ineffective and should not be used (Mueller et al. 2000, 2004; Rath et al. 2004; Jansen 2006; and van der Kooy and Sullivan 2013). This view is unfortunately too simplistic. If we take a closer look at traditional medicines, we have to realise that the chemistry of any traditional formulation consists of two parts (1) the chemistry of the medicinal plant and (2) the chemical influence that the preparation method has on the final formulation. The first aspect has been studied in some detail, and over the years, more than 600 secondary metabolites have been identified and reported for *A. annua* (Brown 2010). In contrast to this, only 37 compounds have been identified in tea infusions or cold water extracts, mainly consisting of caffeic acid derivatives, flavonoids, coumarins and artemisinin (van der Kooy and Sullivan 2013). The influence of the preparation method on the chemistry of the final formulation has not been studied at all. To put this into perspective, we all know that a raw potato tastes different than a cooked potato, indicating that the preparation method has changed the chemistry. What effect will the preparation method have on the chemistry of the final formulation of the *A. annua* tea infusion? The chemical changes that can occur are not only due to the high temperatures involved but also due to water chemistry. Under field conditions, people will use any water available to them to prepare their tea infusions, and we know that water contains a large and variable amount of salts and elements. Any of these salts or elements can react to any organic molecule extracted from *A. annua*. The implications of this have been discussed by Van der Kooy and Sullivan (2013). These chemical changes can possibly have a large effect on the overall activity of the tea infusion. Here, we also have to clearly state that it can potentially have the opposite effect as well—the infusion might become less effective.

In our lab, we recently identified two new compounds in the tea infusion, *cis*- and *trans*-melilotoside, which was the first report of these compounds in any *Artemisia* spp. (Mouton et al. 2013). Figure 14.2 illustrates the chemical structures of some of the compounds identified in the *A. annua* tea infusion. Due to the limited chemical data, we do not yet know which compounds in the *A. annua* tea infusion are responsible for the anti-HIV activity, and the possibility that it might be one of the 37 compounds already identified will be further discussed. We have to keep in mind that we are using in vitro bioassays, which will exclude any prodrugs being identified or any compound whose mechanism of action is different to that targeted in the bioassay. In this section, we will describe what is currently known on the anti-HIV activity of all the identified compounds to date.

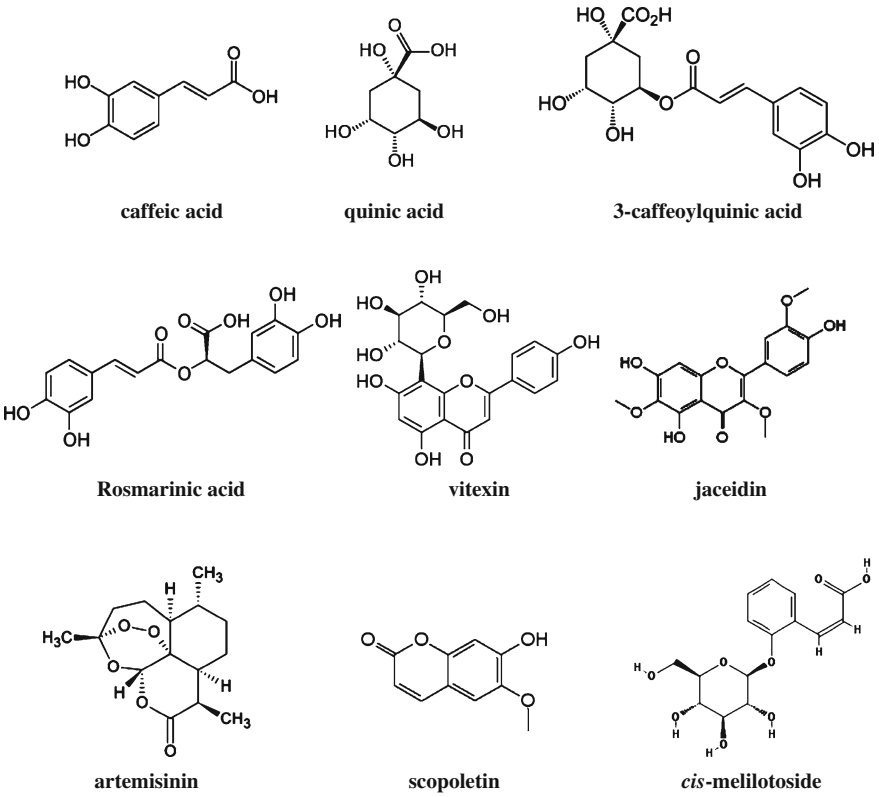

Fig. 14.2 The chemical structures of selected compounds identified in the *Artemisia annua* tea infusion, illustrating the chemical diversity in the tea infusion

14.3.1 Anti-HIV activity of compounds identified in the Artemisia annua tea infusion

Chlorogenic acid derivatives: This group of compounds are widely distributed in the plant kingdom. This fact also makes their biological activity and especially their anti-HIV activity controversial. Almost all plants contain chlorogenic acid and its derivatives, and it would therefore be odd if these compounds are also the main active constituents. Or is this maybe proof for "you are what you eat", indicating that a good balanced diet consisting of fruits and vegetables will fend off disease? Nevertheless, this class of compounds are thought to exhibit good anti-HIV activity with patents covering their use as anti-HIV treatments (Harding et al. 2011; Sun et al. 2008). Cos et al. (2004) have also reported that 3,5-dicaffeoyl-quinic acid does indeed show good activity against HIV integrase, although controversy remains around its potency and activity in vivo. However, our latest finding based on the positive reports from African communities using the

A. annua tea infusion for the treatment of HIV indicates that there is indeed strong anti-HIV activity in the *A. annua* tea infusion (Lubbe et al. 2012) and that this activity can therefore partly be ascribed to the chlorogenic acid class of compounds.

Coumarins: Both coumarins identified in the *A. annua* tea infusion were tested by Wu et al. (2001) against HIV and were found to be inactive. Coumarins are however known for their anticoagulation properties with warfarin being the best example. This possible influence on blood coagulation properties may have an indirect effect on the anti-HIV compounds, although this remains to be shown.

Melilotosides: These relatively rare compounds did show good activity against the diarrhoea-causing pathogens *Entamoeba histolytica* and *Giardia lamblia* (IC_{50} = 12.5 and 16.8 μg/mL, respectively), indicating that the traditional use of *A. annua* for the treatment of diarrhoea might be effective (Calzada et al. 2003). No reports could be found on their activity against HIV, although these compounds will be hydrolysed into their respective *o*-coumaric acids in the digestive tract. The *o*-coumaric acids have not yet been tested against HIV, but Zhuang et al. (2009) have reported that they exhibit some activity against the SARS virus.

Flavonoids: Vitexin, chrysoeriol and cirsilineol were tested by Wu et al. (2001) against HIV replication and were found to be inactive. Piccinelli et al. (2005) also tested vitixin and isovitixin against HIV and reported weak activity. Patuletin glycoside has not yet been studied against HIV, while luteolin-7-O-glucoside and rutin were tested by Lee-Huang et al. (2003) and were found to be inactive. Jaceidin, chrysosplenol D and chrysosplenetin have also not yet been tested against HIV. None off the flavonoids identified thus far in the *A. annua* tea infusion show strong activity against HIV in any in vitro HIV bioassay used. We have to keep in mind that in vitro bioassays will exclude identification of any prodrugs. To put this into perspective, Tao et al. (2007) reported on the strong anti-HIV activity of the sodium–rutin–sulphate complex. This highlights the possibility that salts and elements in water can "activate" common compounds which might otherwise be inactive.

Terpenoids: Artemisinin has been tested against various viruses. Efferth (2008) reported on the broad antiviral activity of artemisinin and its semi-synthetic derivative artesunate. Artesunate showed inhibition of HIV at levels of 600 nM, but no reports on the activity of artemisinin against HIV was given. Jung and Schinazi (1994) reported on the anti-HIV activity of artemisinin, with EC_{50} and IC_{50} greater than 100 μM. Benedikt et al. (2005) have patented artemisinin and some of its derivatives against various viruses. In the patent description, the activity of artemisinin was given to be insignificant against HIV-1 and HIV-2. Lubbe et al. (2012) found artemisinin to be inactive against HIV at a concentration of 25 μg/mL. We therefore have some conflicting reports on the activity of artemisinin against HIV probably caused by the different bioassays used.

14.4 Discussion and Conclusions

From the above discussion on the available literature associated with the identified compounds, we can conclude that it remains unclear which compounds are responsible for the observed in vitro activity and the claimed in vivo activity. The chlorogenic acid family does have some potential role to play in this observed activity, while the coumarins appear to have no direct role to play. The melilotosides have not yet been tested, while some of the flavonoids were tested but did not show a high level of activity against HIV. The concentration of the flavonoids in the tea infusion was also found to be rather low, which furthermore decreases the possibility that this class of compounds are responsible for the activity. If the flavonoids have any role to play, it will be in the form of a prodrug, as Tao et al. (2007) reported on the strong anti-HIV activity of the sodium–rutin–sulphate complex. Artemisinin has been tested against HIV using various bioassays, and the reported activity is somewhat contradictory. It therefore appears that none of the compounds identified thus far can be seen as the main anti-HIV compound. The logical next steps to take would be to perform a full chemical investigation into the tea infusion including the chemical reactions that can take place during the preparation of the tea with various chemicals present in water. It will also be important that the in vitro anti-HIV activity of the tea infusion be confirmed by an independent laboratory in order to decrease the possibility that this result might be a false positive. If the activity can be confirmed, the active compounds should be identified with the normal bioguided fractionation processes. Here, we have to keep in mind that fractionation can lead to the loss of activity and this effect, combined with the difficulties we face with in vitro bioassays, can make this task more difficult than what it appears (Schmidt et al. 2007; Wagner and Ulrich-Merzenich 2009; and Gertsch 2011). To give us a potential lead and to narrow down the possibilities, we can again take a reverse approach. If we look at the chemical structures of existing HIV drugs that are currently being developed or used, is there any similar structure to those compounds identified in the *A. annua* tea infusion? There is one HIV drug, Bevirimat, based on the structure of betulinic acid, a triterpenoid. The Asteraceae family of plants is known to contain a wide variety of terpenoids with various biological activities associated with this group of compounds. *A. annua* has been reported to contain oleanolic acid and other tritepenoids which is structurally similar to Bevirimat. As of yet, none of these compounds have been identified in the tea infusion, but it appears that focussing on the identification of terpenoids in the tea infusion might be a good starting point.

In this chapter, we have highlighted some aspects regarding the drug discovery process and the severe shortcomings with our current approaches. This chapter is not about *A. annua* but rather uses this medicinal plant as an example to illustrate some rather philosophical aspects in our approach to the general health care system in Western countries. The reverse pharmacology approach does indeed hold a key to a faster and more inexpensive development of new medicines, but in order for this approach to be fully developed and exploited, it will unfortunately

take a lot of persuasion. The best way to persuade the powers that be is to provide the scientific evidence of the effectiveness of this approach. And this is just where the problem lies. It will only be supported once the evidence is provided, and in order to provide the evidence, it must first be supported—a classical catch 22.

The cost of medicine plays only a modest role in the overall cost of health care in Western countries. The capitalistic approach to health care has already unfortunately resulted in the exclusion of a large number of citizens in these countries, and it will be interesting to see how governments will deal with this growing problem. Will they shift their focus from sustaining a too expensive health care system or will they start to investigate any and all possible methods to improve the health of their citizens and reduce the cost, even if this means using inexpensive medicinal plants. In this chapter, we used *A. annua* as an example of how quick a potential lead can be found with the use of the reverse pharmacology approach. The scientific investigation into the medicinal properties of this remarkable plant is unfortunately very limited for the reasons stated above. We do, however, know that this plant has been used for hundreds of years for the treatment of various ailments without any reports of serious side effects or toxicity. This plant is currently being used for the treatment of malaria (approved by the WHO) and HIV (ethnobotanical use), and there are also reports that people have started to use *A. annua* for the treatment of various forms of cancer (Anamed). Lui et al. (2011b) have recently shown that artemisinin does indeed show selective action against breast cancer cell lines, and this selectivity should be more than enough reason to study this plant in more detail. We can conclude that the development of new drugs is prohibitively expensive, and that many lead compounds fail during clinical trials. With *A. annua,* we have an uncontrolled clinical trial happening in exactly the right place—in countries suffering the most under the burden of HIV. It is therefore important to put health before short-term profits as this will lead to long-term profits. We are therefore calling for performing an in-depth scientific study into the traditional formulation consisting of *A. annua* for the treatment of HIV, malaria and also certain forms of cancer. This medicinal plant definitely has the potential to have a global impact on our approach to health care, but importantly we also have to be honest. After scientific scrutiny of *A. annua,* it might turn out not to work in the way we expect—only time will tell.

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