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***Artemisia afra,* a treasure chest of new drugs?**

***In vitro* and animal studies? - NO**

Randomized controlled trials? - Maybe

Artemisia afra, a controversial herbal remedy or a treasure trove of new drugs?

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Abstract

Ethnoparmacological relevance: *Artemisia afra* is one of the most widely used herbal remedies in South Africa. This highly aromatic shrub is used to treat various disorders including coughs, colds, influenza, and malaria. Due to the long tradition of use and popularity of *A. afra*, it has been successfully commercialised and can currently be bought from various internet stores and pharmacies. The most notable indication is for the prophylaxis and treatment of *Plasmodium falciparum* infections. In 2013, the Medicine Control Council (MCC) of South Africa banned the sale of *A. afra* for the treatment of malaria because it lacks scientific evidence of efficacy. This resulted in a lawsuit being filed in 2017 against the MCC by an herbal company which claimed that artemisinin was responsible for *A. afra's* antiplasmodial activity. At the time, no scientific literature reported that *A. afra* contained artemisinin.

Materials and methods: This review aims to collate all available scientific literature regarding the phytochemistry and biological activity, focusing on antimalarial activity, of *A. afra* published from 2009 to 2019 and follows on our earlier review, which covered all literature until 2009. All scientific literature in English published between 2009 and June 2019 were retrieved from scientific databases (Scifinder scholar, Web of Science, Scopus, PubMed, Google scholar) and a number of books regarding medicinal plants in South Africa were also consulted.

Results: In the last decade **v**ery few compounds have been identified in *A. afra*, none of which were novel compounds. Based on all the tests that have been conducted using extracts and compounds of *A. afra* in a disparate variety of *in vitro* and *in vivo* bioassays, the results indicate only weak biological activity. The activity of extracts, and in some cases pure compounds, exhibited IC_{50} or MIC values of 1 000 – 10 000 fold

less active than the positive controls. In contrast, and quite surprisingly, two randomised controlled trials were recently conducted (*Schistosoma mansoni* and *Plasmodium falciparum* infected patients) and although criticised based on design, execution, statistical analysis and ethical concerns, showed remarkably positive results.

Conclusions: Pre-clinical *in vitro* and *in vivo* animal experiments failed to yield any promising drug leads. However, if the recent randomised controlled trials can be independently replicated in well-designed and executed clinical trials it might indicate that *A. afra* contain powerful 'prodrugs'. Future research on *A. afra* should therefore focus on reproducing the randomised controlled trials and on artificially metabolising *A. afra* extracts/compounds in order to identify the presence of any 'prodrugs'.

Keywords: *Artemisia afra*, *Plasmodium falciparum*, malaria, artemisinin, randomised controlled trial, prodrugs

Abbreviations: ASAQ, artesunate—amodiaquine; DCM, dichloromethane; HIV, human immunodeficiency virus; IC50, half maximal inhibitory concentration; LD50, median lethal dose; MCC, medicine control council; MeOH, methanol; MIC, minimum inhibitory concentration; PCR, polymerase chain reaction; RCT, randomised controlled trial; SAHPRA, South African health products regulatory authority; TB, tuberculosis

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1. Structure of this review

Since our previous review of *Artemisia afra* Jacq. ex Willd (Liu et al., 2009), a number of recent developments prompted us to review all available literature published since 2009. The two most notable developments were a court case in 2017, where a South African herbal company took the Medicine Control Council (MCC) - now known as the South African Health Products Regulatory Authority (SAHPRA) - to court (https://www.iol.co.za/pretoria-news/malaria-muti-battle-in-court-12030332) after the MCC banned the sale of *A. afra* for the treatment of malaria by declaring it 'undesirable' (https://search.opengazettes.org.za/text/9468?dq=no%2037075&page=2). The MCC also arrested the practitioner and confiscated commercial stock (https://citizen.co.za/news/south-africa/1728107/johannesburg-gp-takes-on-health-department-over-malaria-capsules). The second notable development was a recently published randomised controlled clinical trial (RCT) reporting far superior cure rates of *A. afra* (and *A. annua*) infusions for the treatment of malaria as compared to the artemisinin combination therapy, artesunate—amodiaquine (ASAQ) (Munyangi et al., 2019).

This review will consist of two parts. The first part will cover the general ethnopharmacology, phytochemistry, biological activity and toxicity reported for *A. afra* published in the English language scientific literature since 2009, whilst the second part will focus on *A. afra's* purported *in vitro* and *in vivo* antimalarial activity with special attention and critical appraisal given to the recently published RCT.

2. Introduction and botanical aspects

The genus *Artemisia* (*Asteraceae*) consists of approximately 500 species distributed across several continents throughout the world (Bora and Sharma, 2011; Avula, et al., 2009). *Artemisia annua* L. and *A. absinthium* L. are probably two of the best known species in this family, with the former being the main source of the antimalarial compound artemisinin, and the latter the main ingredient of 'absinthe' or 'bitter', which has been used for centuries in medicinal preparations and alcoholic beverages. In the southern African region, *A. afra* is the dominant species, and although not as well-known as *A. annua* and *A. absinthium*, have an equally long tradition of use, mainly for medicinal purposes.

Artemisia afra is an erect, perennial woody shrub with stems ranging from 0.6m up to 2m tall with a leafy, hairy and ridged stem. The leaves are oval shaped, have a soft texture and exudes a sweet-smelling aroma when crushed. From January until June *A. afra* produces yellow flowers. The plant's silver-white coloured fruit is roughly 1mm in length with a curved shape consisting of three angles. *Artemisia afra* species is found in eastern as well as southern Africa, including South Africa, Kenya, Zimbabwe and Ethiopia and is known by various names across South Africa, including African wormwood (English), "Wilde als" (Afrikaans), "Umhlonyane" (Xhosa, Zulu) and "Lengana" (Sotho, Tswana) (Van Wyk, B-E. and Van Wyk, P. 1997; Van Wyk et al., 2002).

3. Ethnopharmacology of *Artemisia afra*

Artemisia afra is one of the most commonly used plants in traditional medicine in South Africa. The treatment of a wide ranging and unrelated list of health conditions and symptoms indicates that *A. afra* can, or should be considered a panacea. According to Watt and Breyer-Brandwijk, (1962), "the usual preparation is an infusion or decoction, often made syrupy by the addition of sugar, especially when the medicine is for bronchial troubles." It is used to treat coughs and colds, chills, dyspepsia, loss of appetite, stomach-ache and other gastric derangements, colic, croup, whooping-cough, gout and as purgative, flu, headaches, inflammation, gout, sore throat, malaria, diabetes, bladder and kidney disorders, asthma, constipation as well as numerous other health problems. The most common use is the insertion of fresh leaves into the nostrils to clear blocked nasal passages (Van Wyk et al., 2002). Focusing on malaria, Watt and Breyer-Brandwijk, (1962), report that the plant is taken for "fevers and in 'blood-poisoning'" and "the plant is used by the European, the Southern Sotho and the Zulu for measles and other fevers, including malaria." Very little is reported on the toxicity of *A. afra*. Watt and Breyer-Brandwijk, (1962) only states that the oil of *A. afra* is "as toxic as oil of sabine, producing a haemorrhagic nephritis, degenerative changes in the liver and pulmonary oedema after experimental administration to the rabbit."

4. Phytochemical analysis of *Artemisia afra*

In our previous review paper, we tabulated all the volatile and non-volatile compounds that have been identified in *A. afra* (Liu et al., 2009). Since 2009, only a few publications reported on the identification of phytochemicals in *A. afra*. Liu et al. (2010) reported three new phenylpropanoids for this species, and More et al. (2012) identified six known compounds. Venebles et al. (2016) isolated a new isoalantolactone whereas Braünlich et al. (2018) focussed on the main types of polysaccharides found in *A. afra.* They found that it contains mainly the pectin type polysaccharide consisting of arabinogalactan, rhamnogalacturonan and homogalacturonan. One feature that they reported for some of the polysaccharides was the relatively high levels of xylose as one of its monosaccharide constituents. Two new guaianolide sesquiterpene lactones and their antiplasmodial activity was also recently reported by Moyo et al. (2019).

In the past ten years not much work has been conducted on the identification of new bioactive compounds in *A. afra,* which is considering its wide-spread medicinal use, quite disappointing. Table 1 lists all the new secondary metabolites identified in the past decade for this species.

5. *In vitro* and *in vivo* toxicity of *Artemisia afra*

Little work has been done focusing on the toxicity of *A. afra* extracts. In 2009 an *in vivo* study in rats established that acute administration of *A. afra* displayed no toxicity with low potential for toxicity after chronic administration (Ntutela, et al., 2009). Lall and Kishore, (2014) reviewed plant species used for skin care and reported that *A. afra* extracts gave a half maximal inhibitory concentration (IC_{50}) value of 16.95 µg/mL on McCoy fibroblast cell line in a MTT toxicity assay. A 2015 study carried out in mice established an oral median lethal dose (LD_{50}) for aqueous extracts of *A. afra* of 7 500 mg/Kg to 12 000 mg/Kg. Due to the LD_{50} having a value greater than 5 000 mg/Kg, *A. afra* aqueous extracts is considered to be non-toxic (Issa and Bule, 2015). It was found that a sesquiterpene lactone in *A. afra* can cause allergic contact dermatitis in humans (Otang, et al., 2015). Mungho et al. (2018) studied acute toxicity effects of a 70% ethanolic extract of *A. afra* in rats and found it to be non-toxic with LD_{50} values of >5000 mg/Kg.

6. Surveys and general biological activity

A relatively large number of surveys and studies testing *A. afra* extracts in various *in vitro* and *in vivo* bioassays have been conducted over the past decade. Sunmonu and Afolayan, (2010) studied the cardio protective effect of an aqueous extract of *A. afra* in isoproterenol-induced myocardial injured rats. They found that *A. afra* offers some cardioprotective effect. Wintola and Afolayan (2010) conducted a survey of the most common plants used for the treatment of constipation and found that *A. afra* was repeatedly mentioned by traditional healers as being effective. Otang et al. (2012) evaluated the prevalence, perceived benefit and efficacy of herbal medicine (including *A. afra*) in the management of opportunistic fungal infections in HIV/Aids patients. Lubbe et al. (2012) conducted *in vitro* tests of *A. annua* and *A. afra* against HIV, and found that the infusions were active at 2 µg/mL (unfortunately, no follow up studies have yet been conducted.)

Amoo et al. (2012) tested the antioxidant and acetylcholinesterase inhibitory properties of long term stored medicinal plants and found that *A. afra* stored for a 12 or 16-year period, had a significantly higher phenolic content and retained its biological activity. Sunmona and Afolayan (2012) also tested the total phenolic content and antioxidant activity of aqueous extracts of *A. afra* and concluded that it had significant antioxidant activity. Molefe et al. (2012) tested *A. afra* extracts against parasitic gastrointestinal nematodes and found it to be active. Spies et al. (2013) tested ethanolic extracts of *A. afra* against two cancer cell lines and reported IC_{50} values of 18.21 and 31.88 µg/mL against the U937 and HeLa cell lines respectively. The aqueous extract did not show activity at the highest concentration tested (250 µg/mL). Mjiqiza et al. (2013) studied the pulmonary effects of *A. afra* extracts on isolated perfused lungs. They found that nebulised *A. afra* produced the greatest improvements in lung function. Maroyi (2014) conducted a survey of alternative medicines for HIV/Aids treatment in resource poor settings and listed *A. afra* as being used as such. Richter et al. (2014) studied the insect repellent properties of *A. afra* and found the aqueous extract of *A. afra* exhibiting strong insect repellent activity.

Fielding et al. (2015) tested a variety of medicinal plants for antifungal activity (*Botrytis cinerea* in apples) whereas Venables et al. (2016) tested the compound, isolantolactone, isolated from *A. afra* for its mechanism of *in vitro* cell death in HeLa cells. They found that this compound induces apoptosis in a mitochondrial and caspase-dependant manner. Mbokane and Moyo (2018) studied the effect of *A. afra* extracts on the growth and disease resistance in sub-adult fish of *Oreochromis mossambicus*. The survival rate was found to be higher in fish fed with higher concentrations of *A. afra*. Mungho et al. (2018) studied acute toxicity and antihypertensive effects of *A. afra* and found it to be non-toxic with LD_{50} values of >5000 mg/Kg. This corresponds well with Issa and Bule (2015) who reported an LD_{50} value of 9833.4 mg/Kg for the aqueous extract of *A. afra* in mice. A 2018 survey found that *A. afra* is used by certain South African traditional healers to treat rhinitis (Semenya and Maroyi, 2018). Gondwe et al. (2018) studied the anti-inflammatory and anti-nociceptive activity of aqueous extracts of *A. afra* in wistar rats and found that it significantly reduced pain. Falowo et al. (2019) found that *A. afra* slowed lipid oxidation in ground pork during cold storage.

From the above it can be seen that *A. afra* has been tested for a large number of completely disparate conditions. The reported activities can be described as ranging from weak to moderate but due to a lack of follow up studies no firm conclusions can be drawn. Based on the above data we can however conclude that specific extracts (aqueous) appears to exhibit relatively low toxicity. The next section will focus on specific medical conditions because more than one study was published enabling us to provide some critical analysis.

7. Activity of *Artemisia afra* against specific disease causing organisms

7.1 Antimicrobial activity

Buwa et al. (2009) screened *A. afra* extracts (water, ethanol and dichloromethane) against a number of microbial spp. and found that it showed moderate activity against all species tested. Van Vuuren et al. (2010) encapsulated the essential oil of *A. afra* and tested various formulations against four microbial species. They concluded that encapsulation did not improve the bioactivity of the essential oil of *A. afra* whilst other plant species did indeed show improved activity. Suliman et al. (2010) tested the volatile

fraction of *A. afra* in combination with various other plant species in order to determine pharmacodynamic interactions against a range of bacterial species. They found predominantly that additive interactions were present. More et al. (2012) tested six compounds isolated from *A. afra* against a panel of Gram positive and negative bacteria. The crude ethanolic extract exhibited MIC values of between 1.6 mg/mL – 25.0 mg/mL whereas the activity of the most active purified compound ranged between 0.25 mg/mL and 1.0 mg/mL.

Hübsch, et al. (2014) studied the interaction of herbal extracts (including *A. afra*) in combination with antibiotics in order to generate an antimicrobial and toxicity profile. Of the 420 antibiotic:plant combinations tested, 14.29% showed synergistic activity, 7.56% antagonistic, 35.71% additive and 42.44% indifferent interactions. Interestingly, *A. afra* showed antagonistic activity in combination with ciprofloxacin against *Escherichia coli*. The authors conclude that "the majority of combinations were found to have no notable interaction, alleviating some concern related to the concurrent use of these two forms of healthcare." This conclusion can be misinterpreted, especially in light of growing concerns regarding negative herb:drug interactions. The 7.56% antagonistic interactions are indeed low, but by itself already concerning. The more problematic aspect is that only 8 of the 420 combinations were tested for toxicity whilst the claim is made that "none of the notable combinations were found to show toxicity."

Van Vuuren and Muhlarhi, (2017) conducted a comparative analysis testing various plant extracts, including *A. afra*, against a panel of drug resistant microbial strains. They found that the plant extracts showed similar or better activity against the resistant strains as compared to the positive control ciprofloxacin. Elemike et al. (2018) converted silver ions into nano particles in the presence of an aqueous *A. afra* extract. Fourier transform infrared analysis indicated that the silver NP were stabilised and capped by natural products from the extract. They report that the antibacterial activity of these Ag-NP's being higher as compared to the extracts alone.

In general *A. afra* extracts and purified compounds showed weak antibacterial activity. Most commercial antibiotics show activity profiles in the low µg/mL range (depending on bacterial species) whereas all the above studies report activities in the mg/mL range, even for purified compounds. This is roughly a thousand fold less active than for known antibiotics and *A. afra* should therefore be considered to exhibit weak antimicrobial activity.

7.2 Activity against *Mycobacterium* species

Buwa and Afolayan (2009) tested a number of plant extracts against various bacterial species including *Mycobacterium aurum*. They report MIC values of 1.560, 3.125 and 3.125 mg/mL for the water, ethanol and dichloromethane (DCM) extracts of *A. afra* respectively. The positive control, streptomycin, gave an MIC value of 0.00075 mg/mL in this bioassay.

An *in vivo* study was published by Ntutela et al. (2009) who tested aqueous, methanol and DCM extracts of *A. afra* against *M. aurum*. Only the DCM extract was found to be active by inhibiting the replication of *M. aurum* when cultured in the presence of the extracts. Separate dose-dependent studies against *M. aurum* and *M. tuberculosis* gave IC_{50} values of 270 µg/mL and 290 µg/mL respectively. Fractionation yielded an sesquiterpene lactone enriched fraction consisting mainly of artemin and arsubin. The IC_{50} values for this fraction was determined as 1.9 µg/mL and 2.0 µg/mL against *M. aurum* and *M. tuberculosis* respectively. The MIC was determined to be 10 µg/mL.

In vivo mice experiments unfortunately indicated that the sesquiterpene lactone enriched fraction and the DCM fraction did not inhibit mycobacterial replication and the pulmonary and splenic bacilli burdens were comparable to the untreated mice. The final conclusion of this study can unfortunately be misinterpreted, is misleading and does not capture the main results. “This study clearly demonstrates that *A. afra* contains *in vitro* anti-mycobacterial activity, modulates pulmonary inflammation in early mycobacterial infection, and that the mouse experimental tuberculosis model may serve as a useful assay for evaluating the utility of phytotherapy.”

Masoko and Nxumalo (2013) evaluated a number of plant species for their antimycobacterial activity using *M. smegmatis* (ATCC 1441). For the *A. afra* acetone extract they report a MIC of 0.39 mg/mL as compared to the positive control rifampicin of 0.125 mg/mL. For a crude extract to exhibit such promising results as compared to the positive control warrants further investigation. Unfortunately, the authors only screened the crude extracts and did not continue with bioguided fractionation.

Lawal et al. (2014) conducted an ethnobotanical survey of plants used for the treatment of tuberculosis (TB) in selected areas of the Eastern Cape, South Africa. One hundred traditional healers were interviewed and *A. afra* was one of three species most commonly cited as being used for the treatment of TB. Thomford et al. (2015) only mentions *A. afra* as one of the species that is regularly used by traditional healers to treat and manage TB, and Gupta et al. (2017) mentions the anti-TB and anti-HIV activity of *A. afra*. Another review (Chinsembu, 2016) demonstrates how easy *in vitro*/*in vivo* results can be misinterpreted and/or misrepresented. The results of Ntutela et al. (2009) which indicated that *A. afra* showed no *in vivo* activity in mice experiments is presented as "… *Artemisia afra* Jacq. contains *in vitro* antimycobacterial chemical agents that modulate pulmonary inflammation in early TB infection. An organic fraction of *A. afra* reduces replication of *M. aurum* and *M. tuberculosis* in a dose-dependent manner with IC_{50} values of 1.9 µg/ml and 2.0 µg/mL, respectively." No mention is made that no *in vivo* activity was found as compared to the positive controls.

7.3 Artemisia afra in the treatment of diabetes.

Afolayan and Sunmonu (2010) reviewed the medicinal plants, including *A. afra*, traditionally and currently being used as antidiabetics. They followed up on this review with an *in vivo* analysis of an aqueous extract of *A. afra* orally administered to streptozotocin-induced diabetic rats. They report that the extract significantly reduced blood glucose levels and increased the serum insulin levels (Afolayan and Sunmonu, 2011). Another similar study concluded that the *A. afra* extract compares favourably with the hypoglycemic drug, glibenclamide. They also tested different dosages of *A. afra* extracts and concluded that the extracts

compared favourably with the positive control but that some kidney functions might be impaired at higher dosages. The dosages tested were between 50-200 mg/Kg of body weight. This is a high dosage level and translate to a dosage of 3.5 g-14 g for an average person weighing 70 Kg. (Afolayan and Sunmonu, 2013)

Nkobolo et al. (2011) tested a number of plant species including *A. afra* for its *in vitro* alpha-oxidase and alpha-amylase activity. Mohammed et al. (2014) reviewed African medicinal plants and their antidiabetic activity and mentions the study of Afolayan and Sunmonu (2011) in regard to *A. afra's* antidiabetic activity. Another review also mentions *A. afra* as being used for the management of diabetes (Arulselvan et al., 2014)

Issa and Bule (2015) conducted *in vivo* experiments using alloxan induced diabetic Swiss albino mice. They administered extremely high concentrations of an aqueous and methanolic extracts (500-1000 mg/Kg). Blood glucose levels were significantly reduced after the administration of 500 and 750 mg/Kg of the aqueous extract and at 1000 mg/Kg for the methanolic extract. The LD_{50} for the aqueous extract was determined to be 9833.4 mg/Kg.

7.4 Activity against *Trypanosoma* species

Nibret and Wink (2010) tested four Ethiopian *Artemisia* spp. against bloodstream forms of *Trypanosoma brucei brucei* and toxicity against HL-66 cell lines. The IC_{50} for *A. afra* extracts was found to be 77.54 and 25.27 µg/mL for the methanol and DCM extracts respectively. The selectivity index was calculated as 5.08 and 4.87, whilst the positive control had an IC_{50} value of 0.088 µg/mL with a selectivity index of >1 464.00. *Artemisia afra* was found to be more active than the corresponding *A. annua* extracts whereas pure artemisinin showed activity of 35.91 µg/mL and a selectivity index of 2.44.

Mokoka et al. (2011) screened a large number of plant species for activity against *T. brucei rhodesiense*, *T. cruzi*, *Leishmania donovani*, and *P. falciparum*. The leaves of *A. afra* was extracted separately in DCM:MeOH (1:1), DCM and methanol and gave IC_{50} values of 21.9, 9.6 and 15.9 µg/mL against *T. brucei*

rhodesiense respectively (positive control: Melarsopol = 0.004 µg/mL). Against *T. cruzi* the *A. afra* extracts gave IC_{50} values of 54.3, 27.6 and 41.8 µg/mL (positive control: Benznidazole = 0.482 µg/mL)

Naß and Efferth (2018) reviewed the activity of the genus *Artemisia* against *Trypanosomiasis* and reported that not only does *A. annua* and artemisinin inhibit various *Trypanosomiasis* spp. but also other *Artemisia* spp. including *A. afra* (which lacks the sesquiterpene lactone artemisinin).

7.5 Activity against Schistosomiasis

Schistosomiasis (bilharzia) is a neglected tropical disease with few cost-effective treatments. Munyangi et al. (2018) conducted a double blind, randomized, superiority clinical trial (n=800) with three treatment arms; 400 received the standard treatment praziquantel, 200 received an infusion of *A. annua*, and 200 received *A. afra*. *Artemisia* treated patients received 1L/day of a leaf/twig tea infusion divided into 3 aliquots for 7 days with 28-day follow-up. Of the 800 enrolled patients having an average of > 700 *Schistosoma mansoni* eggs per faecal sample, 780 completed the trial. Within 14 days of treatment, all *Artemisia* treated patients had no detectable eggs in faecal smears, a result sustained 28 days post treatment. The authors concluded that; "Both *A. annua* and *A. afra* provided faster effective treatment of schistosomiasis and should be considered for implementation on a global scale"

A letter to the editor about this study was recently published detailing a number of (serious) concerns (Argemi et al., 2019). "... there are several crucial issues regarding its scientific background, design, and statistical methods. These concerns question the scientific validity of the results while raising critical issues regarding ethical aspects." A response to these concerns was also recently published by the researchers (Cornet-Vernet et al., 2019).

8. Antiplasmodial* activity of *Artemisia afra

***8.1 In vitro* activity**

Since 2009 a number of *in vitro* studies were published relating to *A. afra* and its antiplasmodial activity. Liu et al. (2010) tested various extracts of the leaves of *A. afra* against *P. falciparum* and found that the non-polar extracts exhibited weak-moderate *in vitro* activity (8.4 – 12.35 µg/mL) whilst the tea infusion showed no activity at the highest concentration tested (20 µg/mL). The *A. afra* material used in this study contained no trace of the active compound artemisinin. Mokoka et al. (2011) tested 300 plant extracts against a number of parasitic diseases including *P. falciparum*. The MeOH, DCM and MeOH:DCM (1:1) leave extracts of *A. afra* gave IC_{50} values of 13.3, 6.2 and 7.5 µg/mL respectively. This corresponds well with the results of Liu et al., (2010).

Muthaura et al. (2015) tested extracts of numerous plant species traditionally used for the treatment of malaria in Kenya, including *A. afra*. A warm water and MeOH extract of the leaves of *A. afra* gave IC_{50} values of 10.2 and 9.1 µg/mL against the D6 strain, and 4.6 and 3.9 µg/mL against the W2 strain respectively. The warm water and MeOH extracts of the stem bark of *A. afra* gave IC_{50} values of 21.6 and 17.8 µg/mL against the D6 strain, and 4.1 and 1.2 µg/mL against the W2 strain respectively. In comparison they also tested *A. annua* which gave similar IC_{50} values of 12.6 and 4.7 against the D6 strain and 14.1 and 5.5 against the W2 strain. The positive control, artemisinin, gave IC_{50} values of 0.9 and 3.38 ng/mL against these two strains. These results are interesting because this is the first report that the stem bark of *A. afra* was tested, secondly that the water extracts showed appreciable activity and thirdly that the activity profiles of *A. afra* is very similar to *A. annua*, the latter which contains artemisinin.

Moyo et al. (2016) investigated the activity of an acetone extract prepared from *A. afra* against early and late stage gametocytes and found *A. afra* to be active with an IC_{50} value of <10 µg/mL. This work was continued and two guaianolide sesquiterpene lactones were isolated and identified. The IC_{50} of both compounds were given as 10 µg/mL and both were found to be non-toxic against HepG2 cells *in vitro* (Moyo et al., 2019).

The *in vitro* tests that have been conducted since 2009 indicates that *A. afra* does indeed contain non-polar compounds that exhibit weak-moderate antiplasmodial activity. The tea infusion does not appear to show

any *in vitro* activity although a warm water extract (60ºC) of the leaves and stem bark displayed some activity.

8.2 Artemisia afra infusions tested in a randomised controlled trial

Since 2009, only one RCT could be found. Munyangi et al. (2019) conducted a double blind, randomized clinical trial with 957 malaria-infected patients. The trial had two treatment arms with 472 patients receiving artesunate-amodiaquine (ASAQ) and 471 receiving *Artemisia* infusions (248 *A. annua* and 223 *A. afra*). The artemisinin content of *A. afra* was negligible (this is the first report that *A. afra* contains artemisinin), but therapeutic responses of patients were similar to *A. annua* treated patients. Trophozoites cleared after 24 h, but took up to 14 days to clear in ASAQ-treated patients. Day 28 cure rates, defined as absence of parasitemia, were for pediatrics 82, 91, and 50% for *A. afra*, *A. annua* and ASAQ, while for adult's cure rates were 91, 100, and 30%, respectively. Only 5.0% of *Artemisia* treated patients reported adverse effects, compared to 42.8% for ASAQ.

This is an unexpected but also a remarkable result and needs some discussion.

-The first point of concern is the very low reported cure rates for the ASAQ treated patients of only 50% and 30% for paediatrics and adults respectively with 42.8% reporting adverse events. In comparison, Mandara et al. (2019) reported a 100% cure rate (PCR corrected) with no adverse events for ASAQ treated patients in a RCT conducted in the Congo. A meta-analysis (Youdom, et al., 2019) reported cure rates for ASAQ of between 88 to 100%, with adverse events ranging from mild to moderate and not directly attributable to drug intake. The results are thus conflicting. A number of possible explanations can be given. Drug resistance against ASAQ in the Democratic Republic of Congo has very rapidly emerged or there might be an adverse herb-drug interaction occurring in the Munyungi trial. All patients in the ASAQ arm received an *Artemisia* placebo consisting of 0.2g/L. Negative herb-drug interactions includes reduced efficacy and/or increased toxicity which may explain the high failure rate as well as the high levels of reported adverse events in the ASAQ arm.

-There is large variation in the age and gender balance between the groups which indicates that randomisation probably did not occur – normally this will invalidate an RCT.

-No PCR corrections were conducted at day 28 making distinguishing between recrudescence and re-infections impossible and hence cure rates cannot accurately be reported.

-The enrolled patient numbers display some discrepancies. In Table S1 the number of patients is n=1000 whereas in the text the number is given as n=957, with n=943 completing the trial. A letter regarding this RCT has recently been accepted for publication which lists a large number of other discrepancies and ethical concerns (Gillebert et al., 2019 – in press).

8.3 Strength of scientific evidence in clinical research

In clinical research there is different levels of evidence regarding healthcare interventions (Figure 1). The lowest level carrying the least amount of weight is *in vitro* studies, followed by animal experiments. Results from these studies is usually not even considered to be evidence and is called “pre-clinical”. Most work that has been conducted on *A. afra* reports on pre-clinical studies and all of these results should be used as a tool to decide if higher level experiments (in general far more time consuming and expensive) should be conducted. However, none of these pre-clinical publications reported in this review provides strong enough evidence to be able to make recommendations regarding the use of *A. afra* in clinical care.

Caution should however be taken in that pre-clinical experiments suffer from both false positive and false negative results. In other words, various *in vitro* and *in vivo* animal studies may indicate that a specific herb is highly effective and safe, but it may turn out to be completely ineffective or toxic when tested in a RCT (false positive), or, quite rarely, the opposite might occur where an herb is ineffective in pre-clinical studies but a RCT can in turn show a clear beneficial effect (a pre-clinical false negative). The latter is rare because a negative pre-clinical result usually stops any further experiments from being conducted. Unfortunately,

false positive/negative pre-clinical results are quite commonly being misinterpreted and presented as evidence of efficacy and safety especially when industry funding is involved.

However, the gold standard remains to be randomised controlled clinical trials. A large number of clinical trials taken together during a meta-analysis of systematic reviews is considered as Level 1 clinical evidence or the strongest level of evidence. Only two 2 RCT's could be found on *A. afra* and no systematic reviews.

8.4 *Artemisia afra* in court

The herbal company selling *A. afra* made use of very low quality pre-clinical evidence to sell their product over the past decade or longer. Their website was updated around June 2017 to include the results of the RCT that was only published in 2019, erroneously stating that; "For all parameters tested herbal treatments was significantly better than drugs with no side effects whatsoever and 100% success rate". The authors of the RCT declared to have no conflict of interest and yet this commercial company somehow reported the results of this RCT two years before it was published (https://web.archive.org/web/20170622103532/http://www.nordman.co.za:80/artemisia.html**).** What is striking is that capsules containing dried *A. afra* (two capsules for adults, 1 for children weighing 15-45 Kg and a half capsule for children weighing >15Kg) and not infusions, as was used during the RCT, are still being prescribed, whilst this aspect has not been tested in the RCT!

They furthermore claim on their website that; "*Artemisia* is effective both for prophylaxis and treatment of malaria." and that the compound responsible for the claimed efficacy is artemisinin; "According to the latest scientific research artemisinin in its natural form is present in almost all Artemisia species…". This claim was tested and the capsules did not contain any detectable trace of artemisinin (Van der Kooy et al., 2008). A complaint was lodged based on these results at the MCC by the late Prof Roy Jobson (unexpectedly passed away in 2018) and partly due to this complaint the MCC declared the product to be 'undesirable', banned the sale thereof, confiscated some of the company's stock and in 2014 also arrested the general practitioner (https://citizen.co.za/news/south-africa/1728107/johannesburg-gp-takes-on-health-department-

over-malaria-capsules). Even though the company continues to sell this product they also decided to take the MCC to court to overrule their decision to ban the product and to declare the arrest of the practitioner as unlawful. According to the journalist who wrote about this case, it is either still ongoing and/or was settled outside of the courts (personal communication). Be as it may, the company continues to market and sell the product to this day.

9. Conclusions and recommendations

Is *A. afra* a treasure chest of new drugs? Based on this review, the answer appears to be, no. Published research conducted over the last decade have shown that *A. afra* exhibits weak *in vitro* and *in vivo* biological activity. A point of concern is the antimycobacterial *in vivo* mice experiments where the researchers unfortunately concluded that *A. afra* show good *in vitro* activity and failed to conclude that based on the *in vivo* result, people might put their live at risk by forgoing effective treatments by choosing an herbal remedy which was shown to be ineffective *in vivo*.

If we look superficially at the results, we might conclude that *A. afra's* antiparasitic activity looks the most promising and should be the focus of future research. However, if we compare the IC_{50} values of *A. afra* extracts and pure compounds with the positive controls, we find a similar situation, with extracts and purified compounds roughly 1 000-10 000 fold less active than positive controls. Thus, based on all the *in vitro* and *in vivo* experiments we should conclude that given the scarcity of resources no further work should be conducted on *A. afra*.

We believe that this conclusion would have been the correct one was it not for the recently published RCT's. A long list of warranted critique about both these RCT's was recently published and it does indeed raise serious question marks. However, if these RCTs can be repeated in well-designed and executed RCT's by an independent research group and if the results are confirmed, then this could indicate that *A. afra* might contain 'pro-drugs', molecules that only become active after administration and metabolism. To put this in perspective. A compound or a number of compounds in combination should increase their *in vitro* activity

by a factor of 1 000-10 000. This is something that can be tested by conducting artificial metabolism experiments, and something that our research group will aim to do.

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Conflicts of interest

The authors declare to have no conflicts of interest

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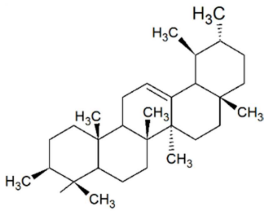
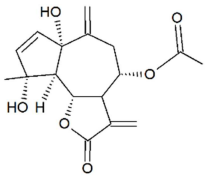
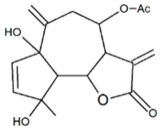
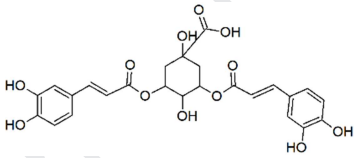
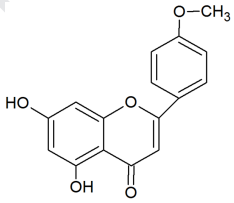
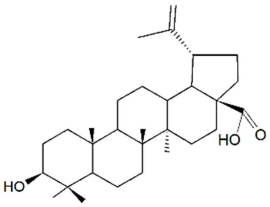
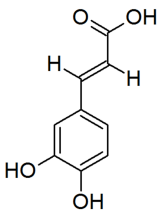
Compound name	Structure	Classification	References
α-amyrin		Pentacyclic triterpene	More et al., 2012
1α,4α-dihydroxybishopsolicepolide		Guaianolide sesquiterpene lactone	Moyo et al., 2019
12α,4α-dihydroxybishopsolicepolide		Sequiterpene	More et al., 2012
3,5-dicaffeoyl quinic acid		Phenylpropanoid	Liu et al., 2010
Acacetin		Flavone	More et al., 2012
Betulinic acid		Pentacyclic triterpenoid	More et al., 2012
Caffeic acid		Phenylpropanoid	Liu et al., 2010

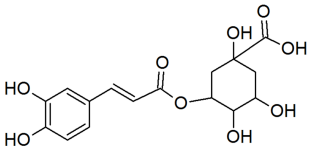
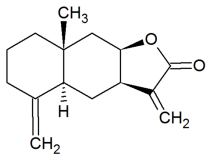
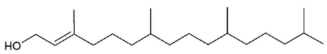
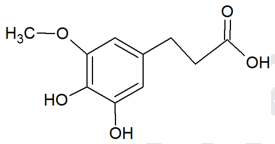
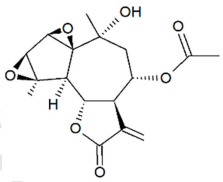
Chlorogenic acid		Phenylpropanoid	Liu et al., 2010
Isoalantolactone		Sesquiterpene lactone	Venables et al., 2016
Phytol		Diterpene	More et al., 2012
Scopoletin		Coumarin	More et al., 2012
Yomogiartemin		Guaianolide sesquiterpene lactone	Moyo et al., 2019

Table 1: Secondary metabolites isolated and identified in *A. afra* in the past decade.

Figure 1. *Artemisia afra* leaves which are most commonly used for the treatment of various ailments.

Figure 2. Pyramid of clinical evidence. The strength increases from low level at the bottom to the strongest level of evidence at the top.