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Use of *Artemisia annua* L. Infusion for Malaria Prevention: Mode of Action and Benefits in a Ugandan Community

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Research Article

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ABSTRACT

Malaria is major public health problem in Uganda endemic in 95% contributing up to 40% of hospital outpatient attendances. Approaches to controlling the disease include; environmental, entomological and medicinal interventions. Some communities use medicinal plants to control the disease. In this paper we report the use of *Artemisia annua L.* for malaria prophylaxis at a Ugandan floricultural farm. We conducted a survey of the farm workers to determine extent of use of *A. annua 'tea',* their clinic attendance patterns and also quantified the levels of artemisinin and flavonoids in *A. annua*. We further tested the effect of artemisinin devoid extract in laboratory animal models. Findings from the survey showed that 84.2% of the managers and 62% of field workers in this farm consumed *A. annua* 'tea' once a week to prevent malaria and related fevers. Clinic attendance due to fevers or symptoms associated with malaria was reduced by 80% while cases of laboratory confirmed diagnosis of malaria reduced by 16.7%. Laboratory test of *A. annua* leaf powder used in community indicated the presence of artemisinin (0.4% to 0.5%) and flavonoids (9% to 11%). *A. annua* extract devoid of artemisinin was found to significantly boost monocyte counts in albino rats

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(p<0.001).The action of these flovonoids could explain the mechanism of prophylaxis of *A. annua* 'tea'. *A. annua* variety or product thereof rich in flavonoids but devoid of artemisinin should be developed and tried for mass prevention of malaria as a beverage or food taken regularly.

Keywords: Artemisia annua; flavonoids; malaria; prevention.

1. INTRODUCTION

Around 60% of the 350 million cases of clinical malaria and 80% of death due to malaria occur in Africa south of the Sahara annually (world malaria report 2005). Malaria and related fevers are major cause of poverty, work absenteeism plus poor performance in schools (Vitor-Silva et al., 2009). On an average, a Ugandan gets between 3 to 6 episodes of malaria annually if no effective prevention measures are used. According to the ministry of health press communication in 2006, Uganda loses US$ 690 million annually due to malaria. This high financial burden is due to the assumption that all fevers are malaria, a situation compounded by limited laboratory testing facilities. It is reported that even where laboratory tests are done, up to 47% of fever patients with negative slides are treated with antimalarials (Nankabirwa et al., 2009). Provision of effective malaria control interventions, such as insecticide-treated nets (ITN), intermittent preventive treatment (IPT) and appropriate case management to all Ugandans is still a big hurdle to climb. A nationwide survey carried out in 2006 by the Uganda bureau of statistics indicated that only 12% of the population used ITNs. The same study reported that 31% of Ugandans still live in abject poverty. Such prevailing conditions in the country encourage individuals and institutions to search for appropriate methods of controlling the disease.

Wagagai (U) Ltd flower farm is one of the communities in Uganda that has been using *A. annua* infusion as a 'tea' for malaria prevention. Most of the farm employees earn less than US $50 per month and therefore cannot afford ITNs or regular treatment for malaria. In an effort to reduce healthcare costs and improve productivity, the farm administration introduced the weekly use of *A. annua* infusion to prevent malaria among the employees in 2006. *A. annua* infusion use for malaria prevention has never been documented. In addition, the active principle in malaria treatment is artemisinin. Due to the short half life of artemisinin (Rath et al., 2004) it may not play a major role in prophylaxis. *A. annua* 'tea' is known to contain flavonoids which have been shown to have antimalarial and antioxidant effects in laboratory studies (Jorge et al., 2010).

In this paper, we report the extent of use, the benefits and possible mode of action of the *A. annua* 'tea' in the control of fevers and malaria among the workers in this farm.

2. MATERIAL AND METHODS

2.1 Study Design

We applied a cross sectional design to collect data among Wagagai flower farm workers between April 2010 and November 2010, followed by experimentation in laboratory animal models.

2.2 Study Site

The study was conducted in Wagagai flower farm located on the shores of Lake Victoria in Entebbe, about 35 km south of Kampala, the capital city of Uganda. Wagagai flower farm was founded in 1998 by Dutch investors and it exports flowers to Europe. The farm employed about 1500 low income workers and provided *A. annua* herbal 'tea' free of charge. The decision to drink the 'tea' is on voluntary basis although the farm encourages all the workers including managers to use it.

Fig. 1. Wagagai (u) Ltd flower farm (Left) located on the shores of *L. Victoria* and *A. annua* L. Plant (Right) in one of their gardens. The farm picture is published with permission from Wagagai (U) Limited.

2.3 Study Material and Identification

A. annua used in this farm is cultivated within the farm and occasionally supplemented from commercial growers when the farm runs out of its own source. In this farm *A. annua* is cultivated only for malaria prevention among the workers. We obtained *A. annua* from a cultivated garden at Wagagai farm, and it was identified by the botanist Nusula M. A voucher specimen NCJ 257 was deposited at the Natural Chemotherapeutics Research Institute, Ministry of Health herbarium. Powder sample of *A. annua* used in this community was tested in laboratory for phytochemical constituents, content of artemisinin and total flavonoids. In the determination of artemisinin yield, 10g of *A. annua* powder was extracted exhaustively using n-toluene solvent by soxhlet. The concentration of artemisinin in the extract was quantified by High Performance Thin Layer Chromatography (HPTLC) method described by Argawal et al. (2009). The total flavonoid content was determined by extracting 10g of *A. annua* powder exhaustively in 80% aqueous methanol (100ml) using soxhlet (Edeoga et al., 2005). The extract was then filtered through Whatman filter paper No 42 (125 mm) and concentrated at low pressures and temperature of 55°C using a rotary evaporator to semi-solid liquid. The semi-solid liquid was then transferred to pre-weighed glass plate and then dried in an oven at 60°C for 8 hours. The weight of the dry extract gave the total flavonoid content in 10g of *A. annua* powder. The procedure to determine the total flavonoid was run in triplicate and range reported. The phytochemical constituents in *A. annua* leaf powder were also identified by qualitative tests described by Edeoga et al. (2005).

2.4 Study Population and Sample Size Determination

All the workers (managers and farm labourers) were included in the survey. To estimate the extent of use of *A. annua*, the benefits and the challenges among the farm workers, a sample size of 369 was computed using the formula; N=[Z2p(1-p)]/ C2 , where Z=value for 95% confidence interval=1.96, p=Estimated proportion of the people in Wagagai taking *A. annua* tea=0.4, C=Confidence interval at ±0.05. This sample size was adjusted for the total population of the farm to generate the final sample size of 296 (Sample size = N/(1+N/P) where P=estimated number of workers in Wagagai farm).

2.5 Sampling and Collection of Information from the Farm Workers

A list of all workers in Wagagai flower farm was provided to the research team by the management. Of the 1515 employees on the list, 19 were managers and 1496 were field workers. We administered a total of 298 questionnaires to all the 19 mangers and to 279 field workers selected by simple random sampling. The participants provided information on their age, sex, years of work at the farm, use of *A. annua*, period of use, purpose of use, other methods of malaria prevention practiced, previous and current history of malaria, including benefits and challenges of *A. annua* 'tea' use.

2.6 Documentation of Clinic Attendance Due to Fevers and Cases of Malaria

During the study period, all farm workers who reported to Wagagai farm clinic with complaint of fever or other symptoms associated with malaria were recorded. Furthermore, each patient provided Information on their employment status and use of *A. annua* for malaria prevention. In the clinic, the policy is that all fever cases are sent to the laboratory for malaria test before any treatment is given. We therefore collected the malaria test results for all fever cases from the clinic during the study period.

2.7 Determination of *A. annua* Extract Effect on Monocyte Levels in Wistar Albino Rats

A study by Turrini et al. (1992) previously reported that monocytes, a group of white blood cells phagocytose red blood cells infected with *Plasmodium falciparum*. In this study *A. annua* dry leaf powder (200g) was extracted in 60% aqueous ethanol exhaustively by soxhlet to extract both artemisinin and flavonoids (Bilia et al., 2006). Ethanol was removed using a Rotary Evaporator at 55°C under low pressures. The aqueous portion was partitioned three times in equivalent volume of petroleum ether to remove artemisinin leaving a portion rich in flavonoids. Confirmation of complete removal of artemisinin from aqueous portion was done using the High performance Thin Layer Chromatography (Agarwal 2009). The flavonoids rich portion was incorporated into starch and dried in an oven at 55°C yielding a dry mass of 4% flavonoid content determined using the method described by Edeoga et al. (2005).

Nineteen Wistar Albino rats were obtained from the same colony. They were weighed and numbered. Six were randomly picked to estimate baseline blood parameters (including monocytes) using Humacount (GmbH, Germany). The remaining 13 rats were then randomized into a control and test group. Each rat in the test group was given a total of 10g of feed mixed with the flavonoid containing extract calculated at 1000mg/kg body weight equivalent to 10% of LD50 for 14 days. The control rats were fed on equivalent weight of rat

feed. Each rat was kept in its own cage and given water ad lib. After 14 days, blood was drawn from the venacava of each rat under chloroform general anaesthesia to determine the monocyte levels.

2.8 Ethical Issues

Permission to conduct the study was obtained from the farm management. For each sampled worker, explanation on the background and purpose of the survey was given first before seeking consent to be interviewed. Participants who gave consent were interviewed by trained field assistants using structured questionnaires read and interpreted for each in the language best understood by the interviewee. Written informed consent was also obtained from workers attending the clinic for fever or other symptoms of malaria. This study also received ethical clearance from the Higher Degree Committee of Makerere University and Uganda National Council for Science & Technology, registration number HS 528. All the animals in the experimental study were treated humanely according to Guidelines for Ethical Conduct in the Care and Use of Animals according to American Psychological Association.

2.9 Data Management and Analysis

All questionnaires were edited for completeness and consistence. Open ended questions were coded before data entry into excel program 2007. Data was then cleaned and exported to Stata version 10 statistical program for analysis. Descriptive statistics (mean, variance, and proportions) were generated. Chi square test was used to establish statistical association between the *A. annua* users and non users. All statistical comparisons were done at 5% level of significance. Since our main outcome was binary (user/non user), we applied logistic regression model in order to establish the relationship between *A. annua* use status and other factors namely, gender, worker's age, years of work and history of malaria. We applied bivariate logistic regression for all the predictors. We further fitted a multivariate logistic regression model to measure the effect of each covariate to *A. annua* use after controlling for other factors. Odds ratios (OR) with their respective 95% confidence intervals were generated for both bivariate and multivariate analysis. For monocyte levels in rats we used paired t-test to compare extract group with control group. Qualitative data was phrased and reported to reflect the meaning stated by the interviewees.

3. RESULTS AND DISCUSSION

3.1 Population Characteristics, Extent of Use and Factors Influencing Use of *A. Annua*

Wagagai flower farm had 1515 workers in total, 19 were farm/clinic managers and 1496 field workers. Of the 298 workers interviewed, 64.1% (191/298) reported routinely using *A. annua* 'tea' for malaria prevention. These comprised of 84.2% (16/19) of the managers and 62.7% (175/279) of the field workers. The average age of the field workers was 29.6±7.1, of which 45.3% were male and 54.7% were female. Only 27.1% (75/279) of the field workers reported use of mosquito nets to prevent malaria. The use of nets in this community compared well with national coverage in which less than 30% of Ugandan population use mosquito nets according to a report in 2006 by the Uganda Bureau of Statistic. This low mosquito net coverage, often poverty related, is one of the reasons for high malaria burden. It is also a driving factor in the search for alternative preventive methods, such as the use of *A. annua*

infusion, in this community. All the workers (managers and field workers) using *A. annua* reported drinking the infusion once a week.

Prophylaxis with a medicine taken on a weekly basis indicates that the said medicine characteristically has a long half-life. Since artemisinin and its metabolite have very short half life (Rath et al., 2004), the once-weekly dose of the 'tea' makes the role of artemisinin less likely in the claimed prophylactic benefit. Other compounds in *A. annua* especially flavonoids, previously reported to have antimalarial and immune modulatory effects, (Jorge et al., 2010) may be responsible for the claimed prophylaxis.

The challenges reported by those using *A. annua* 'tea' related to the taste and the duration of use. Of the 191 workers who reported routinely using the 'tea', 82 (43.0%) reported bitter taste as major problem, while 55 (28.7%) were concerned about safety issues since the 'tea' is to be taken for long durations. The Wagagai farm workers population characteristic and self reported burden of malaria before and following use of *A. annua* are summarised in Table 1.

Table 1. Wagagai farm workers characteristics and reported benefits from *A. annua* use

Characteristics	Mean±SD	Range
Age of workers	29.6±7.1	17-60
Years of employment at farm	3.4±2.0	0 to 6
Years of using Artemisia 'tea'	2.6±1.4	0 to 6
Average malaria frequency/year in those not using Artemisia	0.7±0.7	0 to 2
Average malaria frequency/year in those using Artemisia	0.3±0.46*	0 to 1
Average malaria frequency/year before using Artemisia	4.2± 2.5	1 to1 2

**Workers using A. annua tea reported significantly lower malaria frequency than those not using the 'tea'.*

Bivariate and multivariate analysis indicated that number of years spent at the farm [OR=1.59, 95% CI (1.38, 1.84)] and history of malaria [OR=7.5, 95%CI (4.3, 13)] were significantly associated with *A. annua* use (Table 2). This means that most workers continued to use the 'tea' once they started taking it. The driving factor to use *A. annua* 'tea' as a prophylaxis was found to be the frequency or burden of malaria attacks. Workers with history of high malaria burden were almost 8 times more likely to use to *A. annua* than those with low or no history of malaria burden. High malaria burden was therefore a major reason for a worker to start use of *A. annua* 'tea' in this community. The reported frequency of malaria episode was significantly associated with *A. annua* 'tea' use at only bivariate analysis [OR=1.44, 95% CI (1.27, 1.64)] (Table 2).

Tabe 2. Bivariate and multivariate analysis results of *A. annua* use

Parameters	Bivariate		Multivariate	
	Odds ratio	95% CI	Odds ratio	95% CI
Sex (Female/Male)	1.28	(0.79, 2.09)	1.61	(0.88, 2.93)
Age (in years)	1.06[a]	(1.02, 1.10)	1.04	(0.99, 1.09)
Years of work	1.59[a]	(1.38, 1.84)	1.57[a]	(1.33, 1.85)
Malaria problem before initiating use of *A. annua* (Yes)	7.47[a]	(4.31, 12.96)	4.13[a]	(1.83, 9.29)
Frequency of malaria	1.44[a]	(1.27, 1.64)	1.15	(0.99, 1.33)

[a]*factor significantly associated with use of A. annua 'tea'*

3.2 *A. annua* use in Patients with Fever or Symptoms of Malaria

We documented 100 cases of fevers and/ other symptoms associated with malaria at the outpatient clinic between April 2010 and November 2010. Eighty percent (80) of these were Wagagai farm workers. Of these, majority 64/80 (80%) were not using *A. annua* infusion. While 64.1% of the farm workers used *A. annua* 'tea', it was found that only 20% of fever cases were from amongst this group. This is indicative of the prophylactic benefits reported by the workers interviewed in the survey. The implication of this finding is that if all the workers in this farm used *A. annua* infusion, the burden of malaria and related fevers reported at the clinic would be greatly reduced in this community. Of the 80 fever cases tested for malaria parasites, 58% were confirmed as malaria positive by microscopy. The cases with malaria parasites in the blood were 16.7% less in the workers using *A. annua* 'tea' implying that in addition to reducing clinic attendance due to fevers, it also reduces cases with malaria parasites. An intervention study taking care of adherence to *A. annua* 'tea' use may give a much higher reduction of malaria cases. Therefore it may be necessary to conduct a randomised controlled study to determine the effectiveness of *A. annua* 'tea' in reducing malaria cases, the long term use safety, and possible impact on artemisinin resistance development.

3.3 Phytochemicals Identified in *A. annua*

Phytochemical analysis of the *A. annua* leaf powder showed the presence of alkaloids, coumarins, flavonoids, sterols and triterpenes, tannins, volatile oils, higher fatty acids and reducing compounds. Artemisinin content was found to range from 0.4% to 0.5% while total flavonoid content ranged from 9% to 11%.

3.4 Effect of *A. annua* Extract Devoid of Artemisinin on Rat Monocytes Counts

A. annua extract from which artemisinin had been removed was found to significantly increase the monocyte counts (Table 3). Work done by Mesaik et al. (2009), reported that casticin a flovonoid isolated from *Vitex agnus-castus* significantly inhibited monocyte oxidative burst prolonging their life span and leading to their accumulation in blood. By inhibiting monocyte oxidative burst, the flavonoids prolong the life of monocytes leading to high levels in blood circulation. According to work done by Turrini et al. (1992), monocytes were found to phagocytose red blood cells infected with mature and immature *Plasmodium*

falciparum (malaria parasite) at a rate of 2.3/monocyte and 7.5/monocyte respectively. High levels of monocytes in circulation could possibly result into high clearance of malaria parasites before they mature to cause disease thus improving protection against clinical malaria. This may provide an explanation to the possible mode of action of the *A. annua* 'tea'.

Table 3. Effect of *A. annua* flavonoids rich extract on monocytes in male Wistar albino rats after two weeks of intake

Group	Observations	Mean±SD	95% CI
Control	6	0.07±0.03	(0.05, 0.10)
A. annua Extract	7	0.35±0.09	(0.27, 0.43)*

** *Values for Monocyte levels are X10^3 cells/µl. *Extract significantly elevated monocyte levels (p< 0.001) from baseline.*

The historian Herodotus (484–425 BC) is quoted to have written that the builders of the Egyptian pyramids were given large amount of garlic (*Allium sutiva*), likely to protect them against malarial. *A. sutiva* is now known to contain high concentration of quercetin a flavonoid compound that has both antimalarial and immune stimulatory properties (Saliba et al., 2008). *A. annua* used in this study community contained flavonoids which have been reported to posses both immune stimulatory, antimalarial, anti-inflammatory and antipyretic effects (Sertie et al., 1990, Huang et al., 1993, Bhakuni et al., 2002) and these effects may be contributing to the observed benefits in malaria and related fevers in this study community.

4. CONCLUSION

A. annua has great potential for use in mass prevention of malaria in resource limited settings such as Uganda. The malaria and fever prophylactic effects are possibly due to *A. annua* flavonoids other than artemisinin. *A. annua* variety or product thereof rich in flavonoids but devoid of artemisinin should be developed and tried for mass prevention of malaria as a beverage or food supplement taken regularly.

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