Clinical and Parasitological Effects of *Aspilia africana* (Pers.) C.D. Adams in Fifteen Patients with Uncomplicated Malaria

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This paper reports the findings of a clinical study of a herbal preparation of *Aspilia africana* (Pers.) C.D. Adams against malaria, corroborated by *in vitro* antiplasmodial and cytotoxicity tests. In a non-controlled prospective design, 15 patients with uncomplicated malaria were administered with the herbal preparation and assessed for clinical manifestations of malaria, parasitaemia and global quality of life using the Kanorfsky Performance Scale. Antiplasmodial activity of extracts against the chloroquine-resistant strain of *Plasmodium falciparum* Dd_2 was determined using the [^{3}H]-hypoxanthine radioactive method while cytotoxicity against human urinary bladder carcinoma (ECV-304) and human hepatocellular carcinoma (HepG2) cell lines was determined using the MTT assay. Remarkable clinical improvements occurred 3 to 21 days after initiation of treatment. Forty nine days after starting treatment, all 15 patients had complete resolution of malaria symptoms and were cleared of parasitaemia and attained a Karnofsky Performance score of 100. The petroleum ether/ethyl acetate extract possessed *in vitro* antiplasmodial activity (IC_{50} 30µg/ml) but no remarkable cytotoxicity. The *A. africana* preparation shows potential as an alternative for the management of uncomplicated malaria.

INTRODUCTION

Extensive utilisation of traditional and alternative systems of medicine has spawned a growing interest in their efficacy and safety. This has led to more rigorous assessment of phytotherapeutic approaches in management of diseases including malaria, which is common in tropical and subtropical regions. A number of medicinal plants are traditionally used in the management of malaria [1] and some of them have yielded active constituents currently used alone or in combination as antimalarials.

Aspilia africana (Pers.) C.D. Adams (Asteraceae), a 1.5 meters high woody herb is frequently encountered in traditional African medicine. It is widely distributed in Eastern and Western Africa [2]. The plant is traditionally used as a haemostatic agent, in the treatment of stomachaches and bacterial infections like gonorrhoea. It is purported to alleviate manifestations of inguinal hernia and functional psychosis. [3-4]. The anti-inflammatory activity of *A. africana* has also been demonstrated [5-6]. Decoctions and herbal extracts of the powdered leaves and roots of this plant are used to treat fever, frequently due to malaria. However, while the *in vitro* antiplasmodial activity of *A. africana* and that of a related species, *A. mossambicensis*, have been demonstrated [7-9], there is limited documented evidence for the clinical efficacy of *A. Africana* in management of malaria.

The purpose of this study was therefore to determine the clinical efficacy, *in vitro* antiplasmodial activity and cytotoxicity of *A. africana*.

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MATERIALS AND METHODS

Clinical antimalarial activity of the herbal preparation of *A. africana*

The antimalarial activity of the *A. africana* preparation was tested in a clinical non-randomized and non-controlled prospective study. The decoctions administered to the patients were prepared according to the procedure employed by the traditional healers. For this purpose 5-10 tablespoonfuls of powdered leaves were boiled in 1-2 litres of water, cooled and sieved. The potential benefit of this approach is that it enables clients to access herbs that have been subjected to minimum standardization and packaging requirements.

Selection of study participants

Fifteen adult patients were enrolled in the study. A purposive sampling method was used to select study participants. Inclusion criteria included being a consenting adult at least 18 years old with a low level parasitaemia, 1-9 parasites/100 high powered fields (HPF), presenting with symptoms and signs of uncomplicated malaria. Pregnant women, terminally ill patients, patients with known allergy to the plant under study or similar preparations, those with known renal or liver disease, those on anti-retroviral drugs or anti-cancer treatment and those on any treatment known to potentially interfere with the herbal preparation being administered were excluded.

Data collection

At the time of enrolment, all patients were seen by a study medical doctor, research nurse or clinical officer, to verify clinical diagnosis of malaria and assess the patient for inclusion and exclusion criteria. The history of the participants was taken. In addition, clinical examination and routine laboratory investigations including full haemogram, urinalysis and blood smear for malaria parasites were carried out. Treatment progress, adherence, clinical progression and laboratory investigations were recorded on a follow up form. Severity of the malaria signs and symptoms, Karnofsky performance scale, relevant laboratory investigations, supplementary medications, adverse experiences and decoction accountability were monitored on subsequent visits. The herbal treatment was initiated on day 0 and stopped on day 7. Treatment comprised half a mug of decoction drunk three times a day. Patients however had to report for review on days 3, 7, 21 and 49. At each of these visits, a blood smear for malaria parasites and clinical assessment was done. Patients were followed up on days 4, 8, 22 and 50 using clinical and microscopic assessment of blood for malaria parasites.

The major measurable outcomes were the presence of signs and symptoms of malaria and the change in parasite count relative to the baseline. In addition, the change in global quality of life as determined from the Karnofsky performance scale [10-11] was used as a secondary outcome.

Completed study instruments were collected daily and stored securely at the study site. Weekly editing was done and information entered in MS Access program (Redmond, Washington, United States).The captured data was exported to SPSS (SPSS Inc., IBM Co., Chicago, Illinois, USA) for statistical analysis.

Plant material collection and preparation

Wild growing *Aspilia africana* plants were collected from Central Uganda and identified by Mr. Kakooko, a plant taxonomist. Voucher specimens were deposited at the Department of Pharmacy, Faculty of Medicine, Makerere University. The leaves and upper plant parts of *A. africana* were air-dried and milled. About 300 g of the powder was extracted thrice with 600 ml of ethyl acetate-petroleum ether (1:1) mixture at room temperature for 24 h. The powder was air-dried and similarly extracted with 600 ml of methanol. Finally, aqueous extraction of the air-dried powder was carried out. The extracting solvents were evaporated at 40 °C, 50 °C and 60 °C, respectively, from the extracts under reduced pressure. The resulting residues were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 20 mg/ml and serially diluted to concentrations of 1.56 - 100 µg/ml.

Parasites

The parasite inoculum used was the chloroquine-resistant *Plasmodium falciparum* Dd_2 clone obtained from the Institut fuer Tropenmedizin, Berlin. The parasites were maintained in continuous culture in human red blood cells (A^+) diluted to 5 % haematocrit in RPMI 1640 medium suspended with 25 mM 4- (2 - hydroxyethyl) - 1 - piperazine ethane-sulfonic acid (HEPES), 30 mM $NaHCO_3$ and 10 % human A^+ serum [12].

In vitro antiplasmodial activity

Antiplasmodial tests were performed in 96-well culture plates as described by Desjardins *et al.* [13]. Aliquots of 150 µl of parasitized culture (2.5 % haematocrit and 0.5 % parasitaemia) were exposed to two fold dilutions of test substances. After incubation in the candle jar for 24 h, 0.5 µCi [^{3}H]-hypoxanthine with specific activity of 1 mCi/ml (ICN Pharmaceuticals, Irvine, Calif) were added to each well and the plate incubated for a further 18 h. Cells were harvested onto glass fibre filters with an Inotech cell harvester (Inotech Biosystems International, Inc., Rockville, Maryland) and the incorporated radioactivity in counts per minute (cpm) determined by using a Wallac 1450 Microbeta Plus counter liquid scintillation (Perkin Elmer Wallac Inc., Gaithersburg, Maryland, USA). All tests were performed in triplicate.

The percentage growth inhibition was calculated using the equation:

$$\left[1 - \left(\frac{\text{cpm in treated}}{\text{in untreated cultures}} \right) \right] \times 100$$

The IC_{50} was estimated by interpolation with values above 50 µg/ml being considered inactive [14].

RESULTS AND DISCUSSION

Clinical effects of *Aspilia africana* herbal preparation

At the end of the treatment period, more than 60 % of the patients had no malaria signs and symptoms. Over the study period the overall Karnofsky scores of all the patients improved.

By day 4, >70 % of the patients had no parasites in 100 HPF and by day 21, no client had malaria parasites seen in 100 HPF. This pattern was maintained throughout the follow up period. On days 8 and 50, one patient missed clinical assessment while another one missed parasitological assessment on the day 4 and one was not assessed microscopically on day 22.

Figure 1 shows the clinical symptoms of malaria in relation to parasitaemia and figure 2 depicts change in Karnofsky score over time. It can be seen that the resolution of signs and symptoms of malaria and overall improvement in the quality of life were consistent with the fall in parasite counts during the study period.

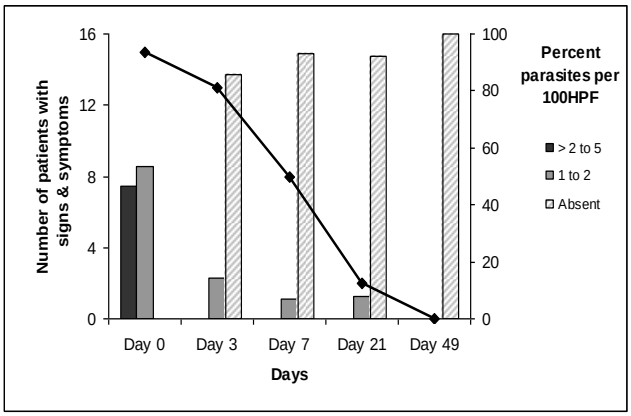

Figure 1: Time course of clinical symptoms and parasitaemia in patients treated with a herbal preparation of *Aspilia Africana*

In vitro antiplasmodial activity

The ethyl acetate/petroleum ether extract of *A. africana* leaf exhibited antiplasmodial activity (IC_{50} 30.3µg/ml) while the methanolic extract was inactive.

Phytochemical screening

Phytochemical screening of *A. africana* whole plant material revealed the presence of secondary metabolites including terpenes, alkaloids, tannins and traces of essential oils.

The insect antijuvenile hormone precocene was also found. Previous studies have reported the presence of alkaloids, saponins, sterols, terpenoid compounds and flavonoids [2,6,9]. *A. africana* var. *africana* and *A. africana* var. *ambigua* have a low content of essential oil content (<0.1% w/w), sesquiterpenes and monoterpenes (limonene and α-pinene).

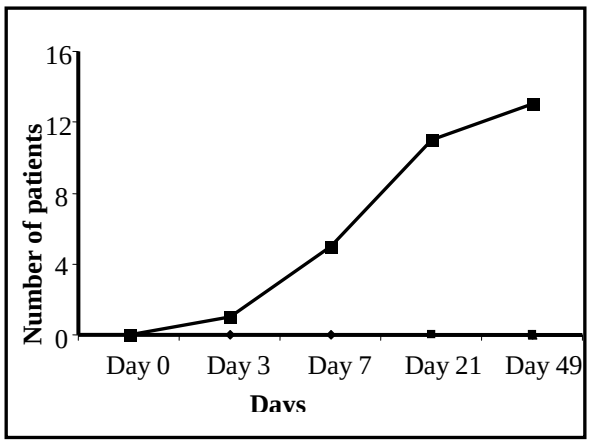

Figure 2: Global quality of life (100 % Karnofsky Score) in patients treated with a herbal preparation of *Aspilia Africana*

CONCLUSION

This preparation shows some clinical benefit and is a potential alternative for the management of un-complicated malaria in areas where the first line treatments of choice are not easily accessible or affordable. More research into appropriate dosage forms and dosage regimens as well as bioassay guided isolation and structure elucidation of antiplasmodial components is necessary.

Table 1: In vitro antiplasmodial activity of leaf and root extracts of *Aspilia africana* against *Plasmodium falciparum* and cytotoxicity against ECV-304 and HepG-2 cell lines

Extract	*P. falciparum*[a] IC_{50} (µg/mL)	ECV 304[b] IC_{50} (µg/mL±SD)	SI ECV/Pf	HepG2[b] IC_{50} (µg/mL±SD)	SI HepG2/Pf
Leaves					
EtOAc/PE	30.3	72.1±2.9	2	136.1±2.5	4
MeOH	>50	118.3±2.4		175.4±5.9	
Roots					
EtOAc/PE	-	83.6±1.9		82.7±1.4	
MeOH	-	131.9±4.7		264.1±17	

- = not carried out, PE - petroleum ether extract, EtOAc- Ethyl acetate extract

ETHICAL CONSIDERATIONS

This study was approved by the Uganda National Council of Science and Technology and additional permission was sought from both the district health authority in Bushenyi and the in-charge of Kabwohe health centre. The study based its safety requirements on World Health Organisation guidelines for research on traditional medicine [16, 17].

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