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Efficacy of herbal remedies used by herbalists in Oyo State Nigeria for treatment of *Plasmodium falciparum* infections – a survey and an observation

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Summary

In the course of evaluating the contribution of phytomedicine to possible drug discovery of antimalarial drugs, an ethnomedical survey of specialized children traditional clinics was done. In the observational multi center study, efficacy of eight different herbal remedies, each consisting of 3-8 ingredients and administered by herbalists were investigated in clients enrolled in the six traditional clinics in Oyo (urban center) and Otu (rural center) of Oyo State, Nigeria. The clients, aged between six months and fifteen years with clinical symptoms of malaria were enrolled in the clinics of the herbalists, as their usual practice. Oral informed consents were obtained from their parents or guardians. Microscopic diagnosis of malaria infection was used to evaluate parasitaemia and validate efficacy of herbal remedies. Results of the analysis showed that, of the163 clients of the herbalists, only 62 (30 from Oyo, 32 from Otu) had microscopically confirmed *P. falciparum* infection. Only results from 54 clients (29/30 (Oyo) and 25/32 (Otu) with *P. falciparum* infection could be evaluated. *Plasmodium falciparum* infection in 88% (23/29) of clients from Oyo responded to treatment with the herbal remedies while cure rate in clients from Otu was 42% (13/25). Parasite densities ranged from 171 to 53,613 parasites/µl blood and 87 to 36,209 parasites/µl blood in patients from Oyo and Otu respectively. The herbalists administered the remedies and *Gossypium arboreum*, *Anarcadium occidentalis*, *Citrus medica*, *Phyllanthus amarus* and *Lippia multiflora* were the main ingredients in the efficacious remedies. The herbalists gave detailed descriptions of each of the 8 herbal remedies proffered. The results confirm the efficacy of two of the eight herbal remedies, thereby validating the role of ethnomedicine as a possible source for the discovery of new chemotherapeutic agents in the treatment of *P. falciparum* malaria.

Keywords: *Herbal remedies, Plasmodium falciparum clearance, herbal clinics, clients, SW Nigeria*

Résumé

Pour évaluer la contribution de la phytomédecine à la découverte des nouveaux médicaments antipaludiens, un controle éthnomedicale dans six cliniques traditionelles dans la province d'Oyo était faite sur un groupe spécifique d'enfants. L'efficacité de 8 rémédes herbal consistant de 3-8 ingredients et administrés par des herbalistes étaient investigués chez ces patients. Les patients agés de 8 mois à 15 ans ayant les symptomes cliniques du paludisme étaient enregistreés dans ces cliniques. L'agréement était obtenue des patients. Le diagnostie microscopique du paludisme était utilisé pour évaluer la parasitémie et la validité de l'efficacité de ces rémédes. Les résultats montraient que sur 163 patients , seulement 62 (30 à Oyo et 32 à Aotu) étaient microscopiquement confirmés positif à l'infection, et seulement 54 patients (20/30 à Oyo et 25/32 à Aotu) étaient évalués. L'infection du *plasmodium falciparum* sur 16% des patients d'Oyo réussis le traitement aux rémédes traditionels alorsque le taux de traitement a Aotu était de 42%. Les densités de la parasitémie variaient entre 171- 53.613 parasites par µl de sang et 87-36.209 parasites /µl de sang à Oyo et Aotu respectivement. Ces principaux ingredients dans ces rémedes inclu *G. Aboreus, A. Occidentalis, C. medica, P amaras et Lippia multiflora* prouvant l'efficacité de ces rémedes. Ces résultats confirmaient l'efficacité de 2 des 8 plantes médecinales ainsi validant leur role en éthnomédecine comme possible source de découverte des nouveaux agents chémotherapeutiques pour le traitement du paludisme.

Introduction

Phytomedicine continues to be valuable for developing antimalarial agents. Medicinal plants used as remedy for malaria and other fevers, remain prominent in various areas of research in malaria, globally [1-7]. Antimalarial compounds derived from different ethnomedicines have been successful in the treatment of both chloroquine sensitive and resistant strains of *Plasmodium falciparum* infections [8-10]. Although many medicinal plants are used as remedies for malaria and other febrile illnesses in Africa have been listed by several authors [11-15], drug development from the plants has been extremely slow. This can be traced to factors, which include non-availability of appropriate

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co-ordination between chemical, pharmacological and clinical aspects of the various investigations.

The study aims at contributing to sourcing of antimalarial drugs from Africa, especially from Nigerian medicinal plants. In fulfilling this goal, validation of herbal remedies in the treatment of *P. falciparum* malaria infection was assessed in six traditional clinics in Southwestern (SW) Nigeria. The observational study was conducted in selected urban (Oyo) and rural (Otu) communities in SW Nigeria. Further to the compilation of medicinal plants used for treatment of fevers and characterization of febrile illnesses in the same region [16], the highlight of the results of a population evaluation and validation of the efficacy of herbal regimen in the treatment of acute uncomplicated malaria is presented.

Participants and methods

The multi center study was observational, the participants being the usual clients of the herbalists in the herbalists' homes/clinics. It was conducted in Otu in Itesiwaju Local Government Area (LGA, rural) and Oyo, Atiba in LGA (urban) in SW Nigeria in July/August 2000 being the rainy season, when there is peak malaria transmission. Six herbal clinics specializing in the treatment of febrile illness were identified for the study. A total of one hundred and sixty three patients (106 urban, 57 rural) participated in the study based on an inclusion exclusion criteria and informed consent necessary for such clinically based study performed thereafter.

Inclusion and exclusion criteria

Inclusion criteria were: Clients of herbalists, compactable with acute uncomplicated malaria, aged between 6 months-15 years; written or verbal informed consent was obtained from the parent or guardian of the client; willingness to comply with the protocol and no antimalarial drug use within 2 weeks of study.

Exclusion criteria were: Clients with any chronic illness which may interfere with evaluation of response to treatment such as respiratory infection; diabetes; sickle cell anaemia; breathlessness and cough; other complicating illness and concomitant use of orthodox antimalarial drugs with herbal remedies.

Conduct of the study

The study participants, clients of the 6 herbalists were male and female out patients. Clinicians, research nurses and investigators (group members) were present when the traditional healers were examining their clients. **Clinical** parameters were taken for all clients. Patients satisfying the inclusion criteria were included in the study at presentation in the healers' clinics after obtaining informed consent. All information obtained were entered into case record forms which were updated at subsequent visits of the clients aged 6 months – 15 years. Six herbalists, 3 from each LGA were selected based on identification by the communities as traditional specialists in treatment of febrile illnesses.

Diagnosis of malaria was presumptive based on clinical symptoms. Thick blood films were prepared from each client prior to treatment, day zero (D0), for microscopic confirmation of *P. falciparum* infection and estimation of parasite density. Each client was treated with herbal remedies prepared and administered in a supervised therapy by the traditional healers on days zero (D0), 3 (D3) and 7 (D7) respectively. Demographic details including history, clinical signs and symptoms were collected by the clinicians and herbalists. The study was conducted in accordance with Good Clinical Practice.

DO: Thick blood films were made, stained with Giemsa to confirm the diagnosis of falciparum malaria. The herbalists gave supervised herbal treatments to patients that had symptoms comparable with falciparum malaria.

D3, D7: Completion of symptom enquiry proforma. Each thick blood film was taken as a follow up, Giemsa stained and screened for malaria parasites. For safety assessment, adverse events were documented at all visits starting from D0 after specific client questioning. The observance of protocol and correctness of documentation were assured by regular GCP-compliant monitoring visits at the study centers 28 days post treatment in each of the study sites.

Treatment and evaluation of efficacy

This was as outlined by the traditional healer. The list of herbs, method of preparation, dosage and route of administration were recorded. Clients were followed up to a period of 7 days. Efficacy of the recipes used for treating microscopically diagnosed malaria included parasite clearance and significant reduction (> 75 % of D0 parasitaemia), recrudescence time and symptom clearance time.

Early and late treatment failure was considered using the R1, R2, R3 and herbal treatment failures was treated with chloroquine or sulphadoxime-pyrimethamine (Fansidar). All data collected were analyzed on the Epi-info soft ware. Data not conforming to normal distribution (example being, parasite density) were log transformed and P values less than 0.05 were considered statistically significant.

Ethical approval

The study protocol was reviewed and approved by the joint ethical committee of the University College Hospital (UCH) and University of Ibadan (UI), Ibadan. Oral informed consent was obtained from the parent/guardian of all participants.

Results

Only fifty-seven of the 163 clients screened had clinically confirmed malaria. The results of the mean parasite count/ µl (P) of blood of clients with clearance of parasites on day O, day 3 and day 7 in both study sites are presented in

Table 1. Mean parasite counts in the treated clients decreased progressively in Oyo and Otu, with the Oyo showing a better clearance level (see Table 1, Fig. 1). The response of patients on Oyo and Otu to herbal treatments on day 3 and day 7 respectively is shown in Table 2. Herbal treatment reduced parasites in 48.3% (14/29 and 25% (5/25) patients in Oyo and Otu respectively on D3, while 44.8% (13/29) and 12.0% (3/25) of the parasites cleared respectively in Oyo and Otu. In Oyo 79% and 52% in Otu showed parasites clearance at D7 (Table 2).

Table1: Mean parasite count of clients (parasite/µl of blood)

Day	Study Site	
	Oyo	Otu
D0	16,660	10,345
D3	3,011	2,764
D7	71	1,060

Figure 1. Clients with parasite clearance on D3 and D7 (%) in study sites

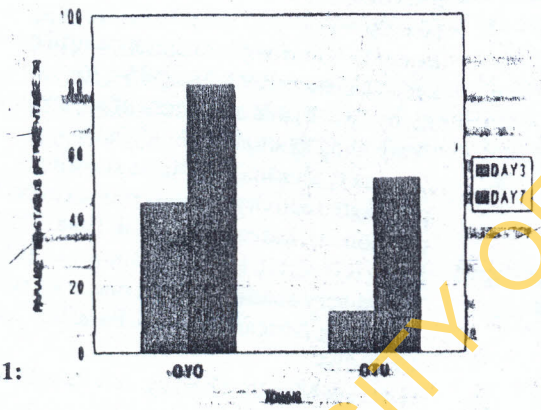

Fig.1:

Table 2: Response to Herbal treatment by Clients of the Herbalists

Parasite level	Site sites (%)			
	Oyo[a]		Otu[b]	
	D3	D7	D3	D7
Clearance	44.8	79.3	12.0	52.0
Reduction	44.8	3.4	36.0	25.0
Failure	6.9	-	52.0	25.0
N/A	-	17.3	-	8.0
Total	100	100	100	100

a. N = 29
b. N = 25

Table 3 shows the remedies proffered by each participating herbalist in relation to the parasite clearance. Herbalist Ebudola (Oyo, 2000) with recipe1 had the most potent herbal remedy with a parasite clearance of 82.6%. Adewale (Oyo, 2000) used recipe wpe 3 and it was not effective in clearing parasites in any of the clients, as shown in Table 3.

Table 3: Herbalists, herbal preparations and percentage clearance of *P. falciparum* parasites in clients in the sites

Herbalists	Herbal remedies	Study sites/Clearance (%)	
		Oyo[a]	Otu[b]
Ebudola	wpe 1	82.6	-
Palengo	wpe 2	17.4	-
Adewale	wpe 3	0	-
Eweje	wpe 4	-	7.6
	wpe 5	-	46.1
	wpe 6	-	7.6
Oladewu	wpe 7	-	23.6
Musiliu	wpe 8	-	7.6
Total	-	100	100

a. **Wpe 1** = *Gossypium arboreum* (l), *Anarcadium occidentalis* (b), *C. medica* (j) - blend and steep in dry gin, take 5 ml thrice daily X 3 days.
Wpe 2 = *Khaya senegalensis*(b), *Mangifera indica* (l) – steep in water overnight, take about 50 ml every morning till symptoms disappear.
Wpe 3 *Cymbopogon citratus* (l), *C. medica* (l & j), *Ananas comosus* (j) - boil in water, take 30 ml once daily X 3 days.
Wpe 4 *Lippia multiflora* (l), *A. occidentalis* (l & b), *M. indica* (b), *Psidium guajava* (l), *C. citratus* (l), *Euphorbia hirta* (l) *C. medica* (j) - boil in water , take 2 tbs full, once daily for 2 weeks.
Wpe 5 = *M. indica* (l & b), *A. occidentalis* (b) *C. citratus* (l), *P. guajava* (l) - boil in water and take 20 ml daily till symptoms disappear.
Wpe 6 = *K. senegalensis* (b), *C. medica* (j) - blend in water, sieve and take 1 tbs, TDS X3 days.
Wpe 7 = *Phyllanthus amarus* (h), *Garcinia cola*, *Aframomum melegueta* (d), *Allium cepa* (l) - boil in water for about 1 h, cool and take 1 tbs full 3 times daily.
Wpe 8 = *Bambusa vulgaria* (l), *C. citratus* (l), *C. medica* (j) - pound in mortar add water, take 40-50 ml TDS.

l = leaf; h = whole herb; j = fruit juice; b = stem bark; d = dried fruit.

b. N = 23
c. N = 13

Herbalists Eweje (Otu 2000) proffered 3 preparations and these cleared *P. falciparum* infections in a total 61.3% clients. In Otu, recipe wpe 5 was the most potent herbal preparation with 8 herbal ingredients by Eweje (Otu, 2000) was more efficacious. All the remedies had the juice of *C. medica* as an ingredient. The most commonly used method of preparation of the recipes was by boiling in water, while the other method described extraction in alcohol (local gin) for a minimum of 12 h. The eight herbal remedies were well tolerated with no reported adverse effect. Clients improved symptomatically over three to seven days. No cases of recrudescence were reported up till Day 28.

Discussion

A herbal preparation in Oyo had greater than 80% reduction indicates that components of the herbal remedy might have been responsible for the parasite clearance. Of the eight herbal remedies (wpe1 - 8), from both study sites, wpe 1 with three herbal ingredients (*Gossypium arboreum* leaves, *Anarcadium occidentalis* stem bark and *Citrus medica* juice), taken as alcoholic extract as shown in Table 3 at a dosage of "5mls" 3 times daily for 3 days, was most effective for parasites clearance (19/23) in Oyo, dosage was the same of age range between 3-12 years, a major demerit of traditional medicine.. *Gossypium arboreum* seed oil is known to contain gossypol, a lipid soluble substance (sparingly soluble in water) with male antifertility, antiproliferative activity *in vitro* against a number of human solid tumour cell lines and *in vitro* and *in vivo* cytotoxicity against CNS tumour cell lines [17], little or nothing is known about the leaves of the plant. The herbalists were therefore advised on the use of the *Gossypium arboreum* leaves until isolation of active ingredients and toxicological evaluation of the plant had been exhausted. The herbalists however argued that the toxicity of the remedy had not been reported over the years hence it was still being used in the particular ethnomedicine.

The results of this study confirm our earlier findings in ethnobotanical surveys in Southwestern Nigeria with regards to constituent herbal ingredients. The use of *Gossypium arboreum* leaves as the main ingredient by Herbalist Ebudola, has informed our interest on further *in vitro* and *in vivo* animal model antimalarial studies on the most efficacious remedy as well as toxicological evaluation of the methanol extract of the plant in animal models. *Lippia multiflora* (Verbenaceae) is an aromatic plant new to the region. According to the Herbalists, the cattle merchants who come in from northern Nigeria probably introduced it to the region. The cows feed on this plant. *Lippia multiflora* leaves have been reported to be used as tea in Ivory Coast and to have antimalarial, hypotensive, fatigue-relieving, diuretic and disinfectant properties [18-20]. *Lippia* leaf essential oils contain sesquiterpenes, sesquiterpene diones and alcohols [21, 22]. *Phyllanthus amarus* synonymous with *P. niruri* L. is highly valued among traditional healers for antimicrobial, antiviral (anti HIV and anti Hepatitis B virus) and antimalarial properties. Previous studies [23-27] on this plant species have furnished ellagitannins, amariinic acid and alkaloids from the polar fractions of the plant extract.

Conclusion

The results from this preliminary study confirm the potential role of ethnomedicine in treatment of falciparum malaria in the region and elsewhere. The herbal remedies evaluated were well tolerated in the clients enrolled in the study. There is need to intensify efforts on bioassay-guided isolation of compounds from these plants that have displayed antimalarial properties in the clients of herbalists in the study region. With further toxicological assessments of the constituent herbal remedies and standardization of dosage, Nigerian phytomedicine may provide a valuable source of antimalarial drugs in the management of malaria in endemic countries.

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