ETHNOPHARMACOLOGY AND TOXICOLOGY OF ANTIMALARIAL PLANTS
USED TRADITIONALLY IN MSAMBWENI, KENYA.

By:

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Abstract

Historically, compounds containing novel structure from natural origin represent a major alternative source for the discovery and development of new drugs for several diseases. This study was undertaken in order to compose detailed documentation on wild medicinal flora used against malaria, existing knowledge, attitudes and practices related to malaria recognition, control and treatment; ethnodiagnostic skill used by the Msambweni community as a lead to traditional bioprospecting and to evaluate the toxicological activity of the crude extracts in brine shrimp bioassay using *Artemia salina* Leach (Artemiidae).

Study I was conducted with herbalists (Traditional Medical Practitioners) to document medicinal plants that are traditionally used by the Msambweni community of Kenyan South Coast to treat malaria, where the disease is endemic. Herbalists were interviewed by administration of semi structured questionnaires in order to obtain information on medicinal plants traditionally used for the treatment of malaria. Focused group discussions held with the herbalists supplemented the interview and questionnaire survey. Twenty six species of plants in twenty four genera distributed in 20 families were reported to be used in this region for the treatment of malaria. Labiatae, Rutaceae and Liliaceae families had each eleven percent of the plant species reported and represented the species that are most commonly used. Thirteen plant species, namely; *Aloe deserti* Berger (Liliaceae), *Launea cornuta* (Oliv and Hiern) C. Jeffrey (Compositae), *Ocimum bacilicum* L. (Labiatae), *Teclea simplicifolia* (Eng) Verdoon (Rutaceae), *Gerranthus lobatus* (Cogn.) Jeffrey (Cucurbitaceae), *Grewia hexaminta* Burret. (Tiliaceae), *Canthium glaucum* Hiern. (Rubiaceae), *Amaranthus hybridus* L. (Amaranthaceae), *Combretum padoides* Engl and Diels. (Combretaceae), *Senecio syringitolius* O. Hoffman. (Compositae), *Ocimum suave* Willd...
(Labiatae), *Aloe macrosiphon* Bak. (Liliaceae) and *Laudolphia buchananii* (Hall.f) Stapf. (Apocynaceae) are documented from this region for the first time for the treatment of malaria.

Study II was conducted with community members to document herbal medicines used in the treatment of malaria as well as the existing knowledge, attitudes and practices related to malaria recognition, control and treatment in South Coast, Kenya. Data was collected using semi structured questionnaires and interviews. A focused group discussion held with the community members, one in each of the study villages supplemented the interview and questionnaire survey. The respondents were found to have a good understanding of malaria and could distinguish it from other disease conditions characterized by increased body temperature. They were also aware that malaria was spread by mosquitoes. Malaria prevalence was high, and affected individuals at an average of four times a year. Community members avoided mosquito bites by using mosquito nets, clearing bushes around their homesteads and burning plant parts to generate smoke. They prevented and treated malaria by taking decoctions or concoctions of traditional herbal remedies. Forty plant species in thirty five genera distributed in twenty four families were used as antimalarials in the study area. Five plant species, namely; *Heeria insignis* Del. (Anacardiaceae), *Rottboelia exaltata* L.F (Gramineae), *Pentanisia ouranogyne* S. Moore (Rubiaceae), *Agathisanthenum globosum* (A. Rich) Hiern (Rubiaceae), and *Grewia trichocarpa* Hochst ex A. Rich (Tiliaceae) are documented for the first time in South Coast, Kenya, for the treatment of malaria.

Study III was conducted with community members to systematically document ethnophytotherapeutic remedies, ethnodiagnostic skills and related traditional knowledge utilized by the Digo community of the Kenyan Coast to diagnose malaria as a lead to traditional bioprospecting. The study was carried out in three Digo villages of Diani sub-location between
May 2009 and December 2009. Data was collected using semi-structured interviews, and open and close-ended questionnaires. A total of sixty (60) respondents (34 men and 26 women) provided the targeted information. The results showed that the indigenous knowledge of Digo community on malaria encompasses not only the symptoms of malaria but also the factors that are responsible for causing malaria, attributes favoring the breeding of mosquitoes and practices employed to guard against mosquito bites or to protect households against malaria. This knowledge is closely in harmony with scientific approaches to the treatment and control of the disease. The Digo community uses sixty (60) medicinal plants distributed in fifty two (52) genera and thirty one (31) families to treat malaria. The most frequently mentioned symptoms were fever, joint pains and vomiting while the most frequently mentioned practices employed to guard against mosquito bites and/or to protect households against malaria was burning of herbal plants such as *Ocimum suave* and ingestion of herbal decoctions and concoctions. The Digo community has abundant ethnodiagnostic skills for malaria which forms the basis of their traditional bioprospecting techniques. They also have abundant traditional knowledge about the causes of malaria and ethnophytotherapeutic remedies.

*Artemia salina*, the brine shrimp larva, is an invertebrate used in the alternative test to determine toxicity of chemicals and natural products. In study IV, the Medium Lethal Concentrations (LC$_{50}$ values) of 170 crude plant extracts and positive controls, cyclophosphamide and etoposide were determined using *Artemia salina*. Out of the 85 organic extracts (Chloroform/Methanol, 1:1) screened for activity against *Artemia salina* larvae, 46 (54%) of the crude extracts demonstrated activity at or below 100µg/ml, and were categorized as having strong cytotoxic activity, 35 (41.2%) of the crude extracts had LC$_{50}$ values between 100µg/ml and 500µg/ml, and were categorized as having moderate cytotoxicity, 2 (2.4%) of the crude extracts had LC$_{50}$ values
between 500µg/ml and 1000µg/ml, and were considered to have weak cytotoxic activity, while 2
(2.4%) of the crude extracts had LC$_{50}$ values greater than 1000µg/ml and were considered to be
non toxic. Approximately 19% (16) of the aqueous extracts demonstrated activity at or below
100 µg/ml and were considered to have strong cytotoxic activity, 39% (33) of the screened
aqueous crude extracts had LC$_{50}$ values between 100µg/ml and 500µg/ml and were considered to
be moderately cytotoxic, 15% (13) of the crude extracts had LC$_{50}$ values between 500µg/ml and
1000µg/ml and were considered to have weak cytotoxic activity while 27% (23) of the aqueous
extracts had LC$_{50}$ values greater than 1000µg/ml and were categorized as non toxic. The positive
controls, cyclophosphamide and etoposide exhibited strong cytotoxicity with LC$_{50}$ values of
95µg/ml and 6µg/ml respectively in a 24 hour lethality study, validating their use as anticancer
agents. In the current study, 97.6% of all the screened organic extracts and 73% of the
investigated aqueous extracts demonstrated LC$_{50}$ values <1000 µg/ml, indicating the presence of
bioactive compounds responsible for the observed toxicity. This calls for in depth in vivo
toxicological studies and chemical investigation for isolation of bioactive compounds responsible
for the observed toxicologic activity. It is concluded that some of the plants used would not make
safe antimalarial drugs, and instead could be a source of novel scaffolds against cancer.

In summary the studies above indicate that many species of antimalarial plants are used by the
Msambweni community to prevent and treat malaria. The good knowledge on the disease by the
study community can be utilized as a lead to bioprospecting of novel remedies accessible to the
rural poor. Majority of the species identified have strong cytotoxic activity in brine shrimp
(Artemia salina) assay, indicating that they could not make safe antimalarial remedies. In depth
studies would now be needed to find the active compounds behind these toxic activities that
could be used as biomarkers in development of anticancerous drugs.