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# Phytochemical investigation on Namibian plants for anti-malaria compounds

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## Abstract

*Malaria is on the decline in Namibia due to interventions by the Ministry of Health and Social Services (MoHSS) and the country is moving towards pre-elimination of the disease. However, barriers such as resistance of the uptake of interventions by "at risk" communities, e.g. lack of treatment seeking behavior for WHO recommended ACT's exist. Some communities in malaria-endemic areas do not accept Western medicine, preferring traditional medicines as prescribed by traditional healers. It is important to balance people's cultural beliefs and practices with the MoHSS's objective of malaria elimination by 2020. To facilitate integration of traditional treatments into mainstream malaria case management, documentation and validation of the treatments to allow their safe and effective use have to be carried out. This study was conducted to document and validate the use of seven plants native to Namibia, targeted on the basis of their indigenous uses which suggest their toxicity to Plasmodium parasites. Crude extracts were prepared using methanol-dichloromethane (1/1v/v) and distilled water at 60 °C. The extracts were further partitioned with chloroform-methanol-water (12/6/1v/v). Preliminary phytochemical screening was performed to detect the presence of selected antiplasmodial compounds. Phytochemical tests revealed the presence of anthraquinones, flavonoids, terpenoids, coumarines, and glycosides; alkaloids and steroids were not detected. Paradoxically, thin-layer chromatography analysis on the crude extracts of the same plants tested positive for all compounds. The presence of these phytochemicals and the data generated support the ethno-medicinal uses for these plants.*

## Introduction

Malaria is one of the major health problems worldwide. An estimation of about 225 million malaria incidences and 781,000 deaths were reported globally in 2009 (USAID, 2011). The causative agent of the disease is a parasite in the blood called Plasmodia. Four species of this genus cause malaria in humans, of which the most deadly is Plasmodium falciparum. These parasites are transmitted through the bite of infected female Anopheles mosquitoes. Fever is the main symptom of cases of uncomplicated malaria (MoHSS, 2005), which can develop into severe malaria as soon as 24 hours after it first appears. Therefore, prompt and appropriate treatment of the disease is necessary in the fight against malaria.

In Namibia, more than half of the population is at risk of contracting malaria, especially

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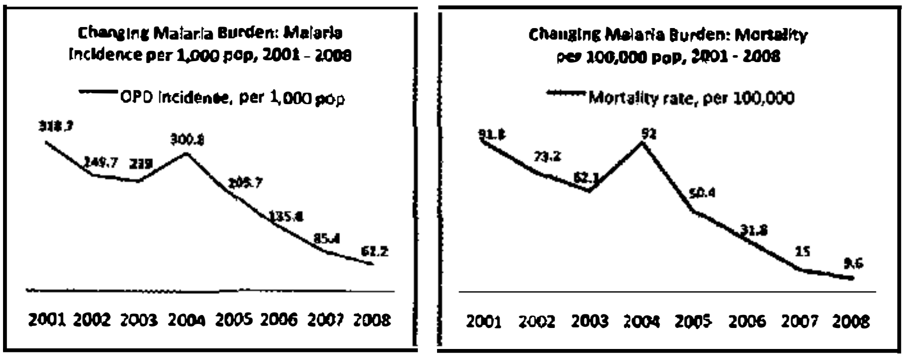
in the northern regions of the country where malaria is endemic. Malaria cases and deaths were abnormally high in the years 2001 and 2004 (MoHSS, 2010a) (Figure 1), as a result of reported cases of high rainfall, lack of vector control and malaria treatment that had become ineffective. Interestingly, malaria deaths have gone down over the past decade with a reduction rate of 90% (WHO, 2010). The incidence of malarial infections has also dropped significantly; hence Namibia is moving towards pre-elimination (>1 case/1000 people) of the disease within its borders (WHO, 2010). Pre-elimination of malaria is the first step towards removing all reservoirs of malaria whilst elimination of the disease means there are no local cases of malaria in Namibia's borders, any cases would be imported from neighbouring countries.

The change in the malaria burden trend is a result of the interventions implemented by the Ministry of Health and Social Services (MoHSS) which include mechanical forms of protection such as the use of insecticide treated nets, intermittent preventive malaria treatment for pregnant women, indoor residual spraying, as well as prompt and appropriate treatment for all malaria cases (WHO, 2010), including the administration of combination drug therapy regimens such as Artemisin Combination Therapy (ACT) (MoHSS, 2009). ACT treatment (either artesunate-SP, artesunate-chloroquine or artemether lumefantrine) shows significant reduction in malarial infections and if lost through resistance, elimination may not be possible (White, 2008).

Government healthcare systems in Southern Africa, including Namibia, are comprised of only Western medical facilities (McGaw, Jager, Grace, Fennel & Van Staden, 2005). However, due to traditional norms and beliefs, and lack of access to such health facilities,




some communities in malaria-endemic parts of Namibia rely on ethnomedicinal plants for their primary healthcare (Lumpkin, 1994).

Figure 1: Trend in Malaria Burden (MoHSS, 2010)

Barriers to the elimination of malaria parasite prevalence exist within the borders of Namibia. These barriers include the resistance of the uptake of interventions by "at risk" communities such as the lack of treatment seeking behaviour for ACTs, the WHO recommended treatment for malaria. It was also found that some communities in malaria-endemic areas do not accept Western medicine preferring traditional medicines as prescribed by traditional healers (Anyinam, 1987; Ajibade, Fatoba, Raheem & Odunuga, 2005). In Namibia such communities seek help from traditional healers before going to healthcare facilities for treatment (Sister Kalumbu, 2011).

It is important to balance people's cultural beliefs and practices with the MoHSS's objective of malaria elimination by 2020. Therefore, to ensure that malaria elimination in Namibia is achieved by 2020, whilst respecting cultural norms; studies on traditional medicine should be carried out to determine the efficacy and safety of all anti-malaria treatments before the promotion of their use. This validation of the traditional treatments will allow their safe and effective use and permit their integration into mainstream case management. Phytochemical investigation is the cornerstone for validating the uses of indigenous medicinal plants; hence, the aim of this study was to identify the presence of selected classes of compounds with anti-plasmodial activity in seven ethnomedicinal plants indigenous to Namibia.

## Plant medicinal value

Plants are of infinite value to less affluent populations. In many malaria endemic countries where modern medicine is inaccessible, plants are used as ethno-medicine to treat disease and a number of ailments. Plants have been used as folk medicine for thousands of years and are still used as an important source of bioactive compounds (Holetz et al., 2002; Willcox, Bodeker& Rasoanaivo, 2004; Ajibade et al., 2005).

Plants synthesize compounds commonly known as secondary metabolites that they use as protection against herbivores (i.e. these compounds act as a deterrent in plants). Secondary metabolites also protect plants from disease-causing agents (pathogens) (Okigbo, Eme & Ogbogu, 2008). These compounds have been shown to produce certain therapeutic effects on the human body (Njoku & Obi, 2009); and may also provide drugs directly such as artemisinin from the Chinese herb Artemisia annua (Carmargo, de Oliveira, Basano & Garcia, 2009), or provide template molecules on which drug molecules can be synthesized organically such as quinine from Cinchona bark (Rosenthal, 2001).

There is a growing demand for traditional medicine; especially in Southern Africa (McGaw et al., 2005). In many African countries where rural people recognize folk medicine as their primary means of healthcare, regardless of the availability and accessibility status of orthodox medical care (Ajibade et al., 2005), these plants also serve as a source of income for them (Hamilton, 2004). Reports also show that pharmaceutical companies mainly synthesize scientific medicine from natural products. Hence, the development of drugs and medicinal agents from plants are necessary in the investigation, prevention and, treatment of infectious diseases such as malaria (Wang, Hao & Chen, 2007).

Namibia is a large country and is sparsely populated with 13 different cultural groups separated by culture and language. Namibia's vegetation was described by (White, 1983) as falling between 3 phytochoria; Zambezi, Kalahari Highveld and Karoo-Namib centres of endemism. The main vegetation types are desert, savannah and woodland which are subdivided into 14 smaller vegetation units (Giess, 1971). This has also allowed the development of traditional medicinal systems that use either different plants to treat similar ailments depending on local availability, or the use of the same plants for different ailments either way resulting in a country with a rich ethnomedicinal heritage which can be tapped into to control malaria. It is not surprising therefore that the areas of richest plant diversity are the same ones who rely more strongly on traditional medicine. These areas also fall into the malaria prone areas and should be a rich source of antimalarial plants. There is decreasing species diversity with decreasing rainfall but endemism shows the opposite trend. This has a bearing on the availability and range of plants for use by local communities (Krugmann, Cole & Du Plessis, 2003). A total of 3159 plant species have been reported to occur in Namibia (Cunningham, 1992; Marshall, 1998; Craven et al., 1997). The Survey of Economic Plants for Arid and Semi-arid Lands (SE PASAL) lists 615 species (19.5%) as being used medicinally.

In view of the difficulties related to the increase in resistance to current drug therapy regimens and the re-emergence of infections, the need for the integration of traditional medicine with modern medicine has been recognized. Integration of traditional medicine as viable treatment options provides an opportunity to introduce novel antimalarial, as well as providing treatment alternatives for communities that do not readily accept Western (allopathic) medicine. The WHO Beijing declaration of 2008 acknowledged the need to integrate traditional medicine into national health systems (WHO, 2008). In the same spirit, the MoHSS Directorate of Pharmaceutical Services is also incorporating traditional or complementary medicine into its updated National Medicines Policy (MoHSS, 2010b). However, concerns about the safety and efficacy of issues prevent traditional medicine from becoming mainstream (Pamela Talalay & Talalay, 2001).

**Documentation and validation of traditional knowledge**

Traditional knowledge (TK) is defined as "a body of knowledge built by a group of people through generations living in close contact with nature" (Traditional Knowledge Sector Paper, 1999). It encompasses all aspects of life within a community and is inherent to the survival and continuity of the community (Krugmann et al., 2003). This knowledge may be useful in the search for new medicine and the development of ethnomedicine from plants that are affordable and accessible to local people. However, this knowledge is only known to the indigenous people, and is passed on orally from generation to generation (Von Lewinski, 2008; Nyota & Mapara, 2008). And because IKS is transmitted orally, it is vulnerable to rapid change disrupting traditional channels of oral communication.

IK is being lost at an increasing rate because of rapid population growth, changes in educational systems, environmental degradation, and development processes all leading to lifestyle changes, modernization and cultural homogenization (International Institute of Rural Reconstruction, 1996). Documentation of TK is therefore important in order to continue its use in providing local solutions or alternatives to Western knowledge; for instilling pride in rural communities about their culture; for the acknowledgment of knowledge holders allowing them to hold the rights so such knowledge, its use and any benefits accruing from it; as well as to validate and promote TK for use in sustainable development.

Validation is an important process which must be carried out to allow the acceptance of traditional medicine as a mainstream alternative to conventional medicine. One of the barriers towards integration of traditional medicine is the safety concerns surrounding its use. Traditional medicines from plants are usually not characterized hence their composition in terms of beneficial compounds and harmful compounds is unknown. Furthermore, questions are raised on whether their use is beneficial or just anecdotal; this may arise from the fact that two people with similar ailments may have different clinical outcomes after using traditional medicines.

Validation may involve the determination of the chemical composition of traditional medicines as well as their biological activity through a process called phytochemical investigation. In some cases, the crude extract of medicinal plants may be used as the treatment or alternatively, the active compounds are isolated and identified for the elucidation of the mechanism of action of the compounds. Hence, research on both mixture of traditional medicine and single active compounds is very important (Joy, Thomas, Mathew & Skaria, 1998). The former allows issues related to toxicity of the traditional medicines to be examined, whilst the latter permits the issue of therapeutic efficacy to be determined. Knowledge of both characteristics not only allows safe and efficacious use of traditional medicines, but also provides a basis for further development of these medicines. Validation of traditional medicines can also be done comparatively with Western medicines with a focus on efficacy, e.g. reverse pharmacology.

## Phytochemical investigation

It has been reported that no or very little information exist on the use of ethnomedicinal plants in Namibia, and studies that have been done on these plants have mostly been done in the northern parts of Namibia (Mapaure & Hatuikulipi, 2007). However, the number of studies is increasing (Chinsembu & Hedimbi (2010); Du Preez et al., (2011); Cheikhyoussef, Mapaure & Shapi, (2011). The medicinal use of these plants is little more than folklore with a small body of evidence showing their utility and safety for medicinal purposes. Safety and lack of efficacy claims are a continual source of controversy that hampers more mainstream use of medicinal plants. The onus of researchers is to generate information on safety and efficacy of these products.

The determination of the chemical composition of traditional medicines from plants is carried out through a process called phytochemical investigation. This process involves the detection of classes of chemical compounds found in preparations of ethnomedicinal plants. Phytochemical investigation for medical purposes must be carried out on plant extracts as they are prepared for use as medicines by traditional healthcare practitioners. However, for research and development phytochemical investigation can be carried out using other methods of extraction to discover the most effective method to isolate bioactive compounds. Standard screens are used in phytochemical investigation which includes a sample known to contain the bioactive compounds expected to be in the extracts prepared. These screens help the researcher to understand herbal medicine and its preparations; aid in the isolation and characterization of the chemicals present in plants; as well as to discover the biological activity of the ethnomedicinal plants.

This research addressed these issues by evaluating and documenting these properties for presentation to the herbal remedies and supplements market. Furthermore, a phytochemical screen for classes of the compounds in the plant extracts is planned and their quantities and this can be used as a reference or pre-packaging quality control tool.

Phytochemical screening was done to evaluate the efficacy of antimalarial plants used in the traditional setting to prevent, cure and/or alleviate symptoms of malaria. As malaria produces a wide range of symptoms, this study focussed mainly on febrile symptoms of the disease; and because symptoms are associated with the release of parasites from ruptured red blood cells into the blood stream, the target for this study was to treat the erythrocytic (blood) stages of the parasites. This study will then create a primary platform for further phytochemical and pharmacological studies.

## Materials and methods

### Collection of plant material

Collection was done in March 2010 and was based on the Ethnopharmacological approach. This approach involves selecting plants based on their ethnomedicinal uses (Tringali, 2001),

p. 394). Indigenous plants used to treat symptoms of malaria by traditional healers and local communities in the Oshikoto region, Engondi Constituency of Namibia were selected for this study (pers. comm., Selvia Nghilifilwa). A permit to collect plant specimens was obtained from the Ministry of Environment and Tourism. The coordinates of the location of the plants were recorded using a GPS device. The scientific nomenclature was confirmed by the National Botanical Research Institute (NBRI) of Namibia. Plant material was air dried for 3-4 weeks at ambient temperature, ground and then stored at -20 °C for further analysis.

**Extract preparation**

Organic and aqueous extracts of selected plant parts were prepared. Half a gram of ground plant material was macerated in 10 ml of water and methanol-dichloromethane in a 1-1 ratio (v/v) respectively. The plant material was soaked for 2 days at ambient temperature using the organic solvent and for the aqueous extract, soaking was done at 60 °C for 2 hours with mixing at 30 minute intervals using a vortex. Thereafter, centrifugation was done for 10 min at 5 000 rpm. Filtration followed, using vacuum filtration for both extracts. Filtrates were collected in 15 ml centrifuge tubes and were then stored at 4 ° C.

**Phytochemical screening**

The plants were evaluated for qualitative determination of known antiplasmodial classes of compounds namely alkaloids (Al), anthraquinones (An), coumarines (C), flavonoids (F), glycosides (G), steroids (S) and terpenoids (T), which were further confirmed by thin layer chromatography (TLC). These tests were carried out using a modified version of the standard protocols described by Njoku and Obi (2009); Nobakht, Kadir and Stanslas (2010); and Fransworth (1966).

**Test for Alkaloids**

Twenty milligram of plant material was soaked in 10 ml of methanol and placed in a water bath for 15 minutes. The suspension was then filtered using Whatman No. 1 filter paper. Six drops of Dragendorff's reagent was added to 1 ml of a mixture that consisted of 2 ml of filtrate and 1 ml of hydrochloric acid (HCl). The observation of an orange precipitate indicated the presence of alkaloids.

**Test for Anthraquinones**

An extract of plant material was prepared by macerating 1 g of the powder form in 10 ml of ether-chloroform for 15 minutes, followed by gravitation filtration. One milliliter of filtrate was then treated with 10 % sodium hydroxide (NaOH). A red coloration indicated the presence of anthraquinones. A dark coloration was assigned +++, a light coloration +, whereas no red coloration -.

**Test for Coumarines**

One gram of ground plant was placed in a small Petri dish, moistened with an aliquot amount of water and then covered with filter paper that was soaked in 10 % NaOH solution. The filter paper was exposed to UV light (360 nm) for several minutes. The appearance of yellow-green fluorescence indicated the presence of coumarines.

**Test for Flavonoids**

Twenty milligram of homogenized plant material was extracted with 1 ml of methanol for 30 min, followed by filtration. Aliquot concentrated HCl was added dropwise to the filtrate. The development of a pink-tomato red indicated the presence of flavonoids (flavons = orange, flavonois = red, flavones = pink).

**Test for Glycosides**

Equal amounts of chloroform were added to 2 ml of the organic extract, which was followed by the addition of 2 ml of concentrated sulphuric acid. The development of a reddish brown color indicated the presence of glycosides in the form of a steroidal ring, which is a glycine portion of a glycoside.

**Test for Steroids**

A methanolic extract was prepared by soaking 500 mg of ground plant material in 5 ml of methanol for 15 minutes, which was then followed by filtration. One milliliter of chloroform was added to the filtrate, and thereafter 1 ml of sulphuric acid. The observation of a yellow-green fluorescent indicated the presence of steroids.

**Test for Terpenoids**

Twenty milligram of ground plant was extracted with 10 ml of chloroform for 15 minutes, followed by gravity filtration. Thereafter, 2 ml of concentrated sulphuric acid and 2 ml of acetic anhydride was added to the filtrate. In the presence of terpenoids a blue-green ring appeared on top of the mixture.

**Thin layer chromatography (TLC)**

Identification of the selected classes of antiplasmodial phytoconstituents was further carried out using aluminum backed TLC plates. The plates were developed with chloroform-methanol-water (12/6/1v/v/v) for both aqueous and organic extracts with positive and negative controls. The plates were then sprayed with staining reagents for selected compounds using standard protocols adopted from Munier, (1953); Gage, Douglas and Wender (1951); Wagner and Bladt (1986); Reznik and Egger (1961); Hoerhammer and Mueller (1954); and Stahl and Kaltenbach (1961).

## Results and discussion

Indigenous plants used for medicinal purposes to treat ailments similar to the symptoms of malaria were identified using local names by traditional healers; voucher specimens were collected and submitted to the NBRI for identification with scientific names (Table 1). This scientific identification was important for accurate documentation as the plants may be known by different names in other regions in Namibia or even within the same region where different dialects are used (Sequeira, 1994). This will assist in any licensing or permissions given by the MoHSS to use the plants as complementary medicines for malaria as they can be issued across regions regardless of the local name. For example, Acacia erioloba (Camel Thorn) which is used to treat fever is called Omuthiya in Oshiwambo and ||Ganab in Nama/Damara. Scientific names also allow the cross comparison of plants and their uses in different areas. This documentation would be important because it allows this knowledge on the plant and its uses to be preserved for future generations (Hedberg, 1993), it also serves as proof of prior art if any benefits resulting from the use of the plants are accrued (Elvin-Lewis, 2007).

Table 1: Indigenous plants used by traditional healers in the Engondi constituency, Ofilu village and their medicinal uses.

| Voucher specimen # | Family name | Specie name | Local name | Ailments |
|---|---|---|---|---|
| CID19 | Vahliaceae | *Vahlia capensis* | Namushinga | Fever |

| | | | | |
|---|---|---|---|---|
| CID20 | Asteraceae | *Nicolasia costata* | Okadimba | Fever |
| CID21 | Bignoniaceae | *Rhigozum brevispinosum* | Omhumakani | Headache |
| CID22 | Pedaliaceae | *Dicerocaryum eriocarpum* | Okalyata | Abdominal pains |
| CID23 | Fabaceae | *Senna occidentalis* | Omutiweyoka | Coughing, chest pain |
| CID24 | Diclidantheraceae | *Lophiocarpus sp.* | Shandamaria | Inflammation |
| CID25 | Fabaceae | *Crotalaria flavicarinata* | Ohamu | Diarrhoea |

Phytochemical tests revealed the presence of anthraquinones, flavonoids, terpenoids, coumarines, and glycosides (Table 2), all of which are anti-plasmodial compounds (Tringali, 2001). Alkaloids and steroids were not detected even though alkaloids were reported to be present in other members of the Asteraceae and Fabaceae families (Ober & Hartmann, 1999) Paradoxically, thin-layer chromatography analysis tested positive for all compounds including those not observed during the phytochemical assays (alkaloids and steroids) (Table 3). This may have been because phytochemicals sometimes have an effect of masking the presence of each other at high concentrations. TLC results in separation of the compounds permitting them to be detected independent of each other (Holetz et al., 2002).

Table 2: Preliminary phytochemical screening for compounds with known antimalarial properties.

| **Classes of antimalarial compounds** | **Plant species** | | | | | | |
|---|---|---|---|---|---|---|---|
| | ***Vahlia capensis*** | ***Nicolasia costata*** | ***Rhigozum brevispinosum*** | ***Dicerocaryum eriocarpum*** | ***Senna occidentalis*** | ***Lophiocarpus sp.*** | ***Crotalaria flavicarinata*** |
| Anthraquinones | - | - | - | +++ | + | - | NT |
| Alkaloids | - | - | - | - | - | - | NT |
| Glycosides | - | - | - | ++ | ++ | - | NT |
| Steroids | - | - | - | - | - | - | NT |
| Terpenoids | ++ | - | - | - | - | - | NT |
| Flavonoids | ++ | +++ | ++ | + | + | +++ | NT |
| Coumarines | ++ | ++ | ++ | ++ | ++ | ++ | NT |

The presence of these compounds which have anti-malaria properties together with their traditional use strongly supports that the plant's medicinal use is rational. Furthermore, knowing the presence of these compounds also addresses safety concerns because side effects of the compound classes are known and they can be coded as potentially having untoward effects that should be looked out for when using the medicinal plants. Additionally, knowledge of the active components of the plant extracts can assist in preparing the plant extracts as some compounds may be enhanced or lost during use of certain preparatory methods. Willcox et al. (2004) found antimalarial compounds to be labile in solvents used for extraction. However, bioassays are usually necessary for unequivocal evidence to say what the effect of the plant extracts have on the Plasmodium parasite.

The methods of preparation also have an impact on the presence and the quantity of the anti-malaria compounds because of solubility issues, aqueous versus organic and the polarity of the solvents (Table 3). Whilst the pattern for finding the compounds was similar, the abundance varied with organic solvents showing more activity of the compounds. This is consistent with other studies which illustrate that plant phytochemicals are more soluble in organic solvents (Willcox et al., 2004).

Table 3: TLC analysis of aqueous (AE) and organic (OE) plant extracts fractionated using chloroform:- methanol-water (12/6/1v/v/v) for the presence of alkaloids, anthraquinones, coumarines, flavonoids, glycosides, steroids and terpenoids.

| Species | Extract | Chemical profiling | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Al | An | C | F | G | S | T |
| *Vahlia capensis* | AE | + | - | ++ | - | ++ | + | - |
| | OE | + | - | ++ | - | +++ | + | - |
| *Nicolasia costata* | AE | + | - | + | - | ++ | - | + |
| | OE | + | - | + | - | +++ | - | + |
| *Rhigozum brevispinosum* | AE | + | - | ++ | - | ++ | - | + |
| | OE | + | – | +++ | - | +++ | + | + |
| *Dicerocaryum eriocarpum* | AE | + | + | ++ | ++ | ++ | - | + |
| | OE | ++ | ++ | ++ | ++ | +++ | - | + |
| *Senna occidentalis* | AE | + | ++ | + | - | ++ | - | + |
| | OE | + | ++ | +++ | ++ | +++ | + | + |
| *Lophiocarpus sp.* | AE | + | - | + | ++ | ++ | - | + |
| | OE | + | + | +++ | ++ | +++ | + | + |
| *Crotalaria flavicarinata* | AE | +++ | + | ++ | ++ | ++ | + | + |
| | OE | +++ | - | +++ | ++ | +++ | + | + |

Key: +++ present, + weakly present, - absent

Interestingly, most medicinal plant preparations are done in aqueous solvents (water). Whilst it is interesting to observe the effect of organic plant extracts and to describe them, perhaps it is more pertinent to focus on the traditional preparation. This is because extraction changes the presence and abundance (quality and quantity) of phytochemicals in the medicinal treatment and as such changes the very nature of the treatment itself (Jordana, Cunninghama & Marles, 2010). The presence and the abundance of the compounds may be the difference between whether the plant will be medicinal or toxic as in the case for Senna occidentalis which though medicinal has been reported to be toxic when eaten by cattle (Orech, Akenga, Ochora, Friis & Aagaard-Hansen, 2005).

## Conclusion

Seven plants used as traditional medicines for ailments similar to the symptoms of malaria were validated by phytochemical analysis. The phytochemical tests and TLC analysis carried out showed the presence of seven antimalarial compounds tested. Previous studies found a direct correlation between the presence of phytochemicals and the ethnomedicinal uses of plants. Therefore, the data suggests that the seven plants have potential antiplasmodial activity warranting further investigation for biological activity against malaria. This study also shows that it is possible to balance people's cultural beliefs and practices with the MoHSS's objective of malaria elimination. More studies involving documentation and validation of ethnomedicinal treatments should be carried out; in addition studies on the antiplasmodial activity of the plant extracts and their mechanism of action of plant extracts should also be conducted to allow the integration of traditional treatments into mainstream malaria case management.

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