Phytochemical Analysis and *In Vitro* Anti-plasmodial Activity of Selected Ethnomedicinal Plants Used to Treat Malaria Associated Symptoms in Northern Namibia.

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Abstract

The search for biologically active compounds from plants is of importance for discovery of novel medicine against infectious diseases such as malaria. The aims of this study were to identify ethno-medicinal plants that are used to treat malaria-associated symptoms in traditional settings in northern Namibia; to detect and quantify the presence of major anti-plasmodial phytochemicals to evaluate the anti-plasmodial activity against *Plasmodium falciparum* 3D7A strain of the extracts of lead plants. Ten plants were identified through a survey conducted by the Multidisciplinary Research Centre at the University of Namibia. Nineteen methanolic extracts from 10 selected ethno-medicinal plants were prepared and analyzed using Thin Layer Chromatography (TLC) to detect classes of anti-plasmodial phytochemical compounds. Furthermore, total phenolic and alkaloids were also quantified. Furthermore, *in vitro* anti-plasmodial activity of crude methanol and aqueous extracts at 5, 10 and 50 μg/ml was evaluated against *P. falciparum* 3D7A strain at 1% parasitaemia and 2% hematocrit after 48 hours. The study revealed the presence of at least one of the major anti-plasmodial classes of compounds such as terpenoids, alkaloids, anthraquinones, flavonoids and coumarins in all the plant extracts analyzed. Out of 10 plant species, *Mundulea sericea*'s shoots indicated the presence of all five major anti-plasmodial phytochemical. The plants species studied show a high estimate of total alkaloids (0.37- 21.21 μg/1g of extracts) and phenolics (99. 58 - 444.07 GA/E μg/ml). Methanol extracts of *M. sericea, D. mespiliformis* and *Cyphostemma* spp showed the best anti-plasmodial activity with range of IC_{50} values from 3.179 to 3.523 μg/ml. The presence of anti-plasmodial phytochemical classes of compounds and high *in vitro* anti-plasmodial activity of selected plants extracts support the further investigation of these plants, as potential sources of novel anti-plasmodial compounds for malaria drug discovery.

Keywords: Phytochemical analysis, Namibia medicinal plants, *M. sericea, D. mespiliformis,*

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Cyphostemma spp

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1 Introduction

The use of plants for medicinal purposes has existed for centuries and is still important for primary healthcare even at a time when modern medicine is widely accepted and used. According to the World Health Organisation (2008), more than 80% of people living in developing countries still depend on herbal medicine to treat common diseases including malaria. People prefer traditional medicine because of wide public acceptance of it being safe and natural with minimal side effects, as well as being easily and cheaply accessible compared to allopathic medicine (Wilcox et al. 2011). Through prolonged usage and experience, indigenous communities had established and correlated the therapeutic effects of plants species to particular disease symptoms. This knowledge has resulted in increased usage of plant based pharmaceuticals to treat common diseases. In particular, 25-50% of mainstream medicines developed directly from plants have shown minimal side effects (Kamalesh & Vickrantsinh 2010). Hence, ethno- medicinal plants are now centre stages for research and development of novel medicines for challenging infectious diseases including malaria.

Anti-plasmodial medicine plays a critical role in malaria case management because prompt and efficient treatment of malaria is required for total clearance of the parasites to resolve disease symptoms. According to Wink (2010), ethno–medicinal plants have always been sources of anti-plasmodial drugs. Two important used anti–plasmodials owe their origin from ethno medicinal plants; quinine a basic alkaloid which is the parent molecule of quinine based drugs such as mefloquinine, chloroquine, isolated from Chinchona bark, a Peruvian ethno medicinal plant (Achan et al. 2011) and artemisinin a sesquiterpene lactone (Brown 2010), which constitute the active component of artemisinin based drugs such as artemether (White 2008). The latter, was isolated from *Artemisia annua*, a Chinese ethno medicinal plants (Miller & Su 2011). Artemisinin Combination Therapy (ACT)'s are currently considered the most effective anti-malarial medicines and are recommended as first line treatment by the World Health Organization (Youdom et al 2010). However, resistance to *P. falciparum* has been reported at the Thailand-Myanmar and Thailand – Cambodia borders (Dondorp et al. 2010; Lin et al. 2010). It has been reported that ACT resistance has spread to Africa as well (Mita et al. 2009; Phyo et al. 2012). Clinical reports show that treatment of malaria with ACT is prone to parasite recrudescence (Dondorp et al. 2012). In addition to reducing sensitivity to ACTs; there is concern about the lack of effective anti–plasmodial medicines (Gelband & Ramanan 2009). Therefore, lack of new malaria medicines and reduced sensitivity, the precursor of resistance highlight the need to search for new medicine for malaria including from natural resources with novel and diverse mode of actions.

In Namibia, over 1000 plants are known to treat fever and other malaria-associated symptoms, in traditional setting (Von Koenen 2001). This represents the enormous potential of

the Namibia flora for bioactive chemicals with possibly unique anti-malarial properties as monotherapies or in combination (Rasoanaivo et al. 2011). Little research has been conducted to understand the bioactive component of ethno medicinal plants for the purposes of safety and evaluation of anti-plasmodial activity of ethnomedicinal plants for the purpose of efficacy and documentation. Medicinal plants tinctures are a complex mixture of phytochemicals that may work individually, in synergy or additively alleviate malaria symptoms (Deharo & Ginsburg 2011).

For this reason it is very important to identify the phytochemical content of herbal medicines to assess functional chemicals that are have shown anti-plasmodial compounds in their respective extracts. This article reports on identification and quantification of major anti-plasmodial classes of phytochemicals and *in-vitro* anti-plasmodial activity from selected Namibian plants species. The phytochemical properties correlate to the anti-plasmodial activities of these plants and may indicate potential for development of the plant extracts as new malaria medicines.

2 Materials and Methods

2.1 Collection and preparation of plants materials

Plants used to treat malaria-associated symptoms were identified through an ethno botanical survey conducted by the Multidisciplinary Research Centre at the University of Namibia. Selection of plants used in this study was based on them belonging to a family associated with malaria and / or fever use and the abundance of species in malaria endemic areas in Namibia. A research permit was obtained from the Ministry of Environment, Namibia and plants were collected from Omusati and Ohangwena regions in Northern Namibia. Voucher specimens of all plants were prepared and the taxonomic identity of the plants was confirmed by National Herbarium of Namibia at the Namibian Botanical Research Institute (NBRI) in Windhoek. Plants leaves, roots and bark collected for analysis were cleaned with water before being air dried at ambient temperature. The plant parts were pulverised, packed and stored at -20 °C till required for extraction.

2.2 Preparation of extracts

The pulverised plant materials (20 g) were macerated at ambient temperature for 72 hours in 400 ml of methanol for organic extracts then gravity filtered using Whatmann No. 1 filter papers. The aqueous extracts were prepared the same way as the organic, with the exception of boiling the mixture in water bath at 70 °C for 5 hours. The extracts were then dried by rotary evaporation before lyophilisation with a freeze dryer. Finally, the dry extracts were weighed and stored at -20 °C till further analysis.

2.3 Thin layer chromatography

Thin Layer Chromatography (TLC) analysis was conducted using modified methods of Harbone (1998). Dry methanol extracts (0.1 g) was reconstituted in 1 ml methanol and spotted on silica gel coated plates (Merk, silica gel 60 F_{254}), using thin capillary tubes. The chromatograms were developed into a tank using solvent systems as shown in Table 1. After, development they were sprayed with prepared staining reagents as shown in the Table 1.

Table 1: Thin Layer Chromatography (TLC) analysis protocol of plants species used to treat malaria in Namibia's malaria endemic regions.

Phytochemical compounds	Mobile phases	Staining reagents	Colours under UV	Colours after staining
Alkaloids	methanol-con ammonium hydroxide (200:3)	Dragendorrf reagent	red & brown	red & brown
Coumarins	hexane-ethyl acetate (4:1)	10% methanolic potassium hydroxide	blue, violet, brown & yellow	blue, violet, brown & yellow
anthraquinones	ethyl acetate: methanol: water (100:17:13)	10% methanolic potassium hydroxide	purple, brown & red	purple
Flavonoids	butanol: acetic acid: water (4:1:5)	1% methanolic aluminium chloride solution	blue & yellow- brown	blue & yellow
Terpenoids	hexane: ethyl acetate (17:3)	Liebermann- Burchard reagent	purple	purple

2.4 Quantification of major anti-plasmodial classes of compounds

2.4.1 Total alkaloids quantification

Total alkaloid content was determined by the method of Shamsa et al. (2008). Briefly plant extracts (1.0 g) were dissolved in 2.00 ml of 2 N hydrochloric acid and then gravity filtered with Whatman No.1 filter paper. The mixture (1 ml) was washed twice with chloroform then the pH of the solution was adjusted to 7.0 using 0.1 N sodium hydroxide. Five millilitres of bromo cresol green solution and an equal amount of phosphate buffer were added to this solution. The mixture was vigorously shaken and extracted with 1, 2, 3, and 4 ml chloroform. The, absorbance of chloroform extracts was measured in triplicate with a GENESYS 20 visible spectrophotometer at 470 nm. Quinine hydrochloride dihydrate a quinine alkaloid was used as a standard at 5, 10, 15, 20 and 25 μg/ml. Total alkaloids were expressed as μg/1g extracts which is microgram per 1 gram of dry plant extracts used.

2.4.2 Total Phenolics quantification

The total phenolics content of plants was determined by using Folin Ciocalteu assay method (Mayank 2011). Briefly, dry and pulverised plant materials (1.00 g), were dissolved in absolute methanoland left overnight prior gravity filtration with Whatman No. 1 filter papers. To 1 ml of the plant's extracts, 1 ml of 10 fold diluted Folin Ciocalteu phenol reagents was added and shaken with 2 ml of 1% (w/v) sodium carbonate. The mixture was incubated for 90 minutes at room temperature and the absorbance was measured triplicate at 765 nm using a GENESYS 20 visible spectrophotometer. Gallic acid a phenolic compound was used as standard at 5, 10, 15 and 20 μg/ml. Total phenolic content was expressed as GA/E μg/ml which Gallic acid equivalent per microgram per ml of dry plants extracts.

2.5 *In vitro P. falciparum* 3D7A culturing and assay

Plasmodium falciparum 3D7A (chloroquine sensitive) strain was used for this study. The entire process of culturing took place in bio safety level II cabinet. Parasites were maintained in continuous culture in the Malaria Research laboratory of the University of Namibia using modified method of Trager and Jensen (1976). Briefly, parasites were cultured on human erythrocytes (blood group O^+ obtained from donors) in RPMI 1640 medium supplemented with 10% human sera, 20% aqueous D^- glucose, 1 M sodium hydroxide and 0.25% gentamicin. The culture was performed in 75 cm^2 sterile flasks containing 10 ml of the culture media with 2% hematocrit and gassed with 90 % N_2, 5% CO_2 and 5 % O_2 and incubated at 37 °C. The parasitaemia was maintained daily between 1 and 4 % by renewing the supplemented RPMI 1640 and adjusting to 2% hematocrit with fresh human erythrocytes (blood group O^+). Dilutions were done using erythrocytes when the parasitaemia exceeded 4%. For that, microscopic observations of prepared thin blood smears fixed with methanol and stained with 5% Giemsa stain in phosphate buffer at (pH 7.1–7.20) were performed daily.

2.5.1 Stock solution preparation

M. sericea (roots and shoots), *D. mespiliformis* (leaves and roots) and *Cyphostemma* spp, whole plants, were selected for anti-plasmodial bioassay based on the presence of the highest abundance of anti-plasmodial phytochemicals classes of compounds tested and high estimate of total phenolics and alkaloids content. Stock solutions of the crude extracts were prepared at 500μg/ml. Lyophilized aqueous and methanol extracts were dissolved in deionised water and Dimethyl sulfoxide (DMSO) respectively. Stock solutions of the artemether lumefantrine (positive control) were similarly prepared in DMSO. The stock solutions were then diluted 1000 folds in the culture medium (RPMI 1640). All stock solutions were sterilized by filtration through 0.22 μm syringe microfilters. The desired concentrations (i.e. 5, 10 and 50 μg/ml) were then obtained from the second dilution in culture medium.

2.5.2 Anti-plasmodial bioassay and assessment

The anti-plasmodial assays were carried out in triplicates on 96- well flat-bottomed micro-culture plates at 1% parasitaemia and 2% hematocrit. The plant extracts were then diluted in the culture medium. The final volume in each well was 100μl. Positive control (artemether lumefantrine), negative control (0.5% DMSO and water) and non treated culture were also included in the assay. The plates were placed in a culture chamber, gassed with gas mixture of 90% N_2, 5% CO_2 and 5% O_2 and incubated at 37 °C for 48 hours.

Parasitaemia of all treatments and controls after 48 hours of incubation was analysed by compound microscopy at 100X magnification. 100 uninfected erthrocytes were counted in 10 different microscopic fields along with infected erthrocytes. Parasitaemia reduction was expressed as percentage of total infected erthrocytes over the total uninfected erthrocytes for each smear. The averages for each treatments and controls were calculated. The IC_{50} values were determined by linear regression of the dose response curve of each plant extracts at 48 hours (Célinea et al. 2009).

Table 2: List of plant species and their putative malaria ethno-medicinal uses at the Northern Namibia.

Families	Plant's names	Local names in Kwanyama	Specimen numbers	Malaria related Ethno medicinal uses
Fabaceae	*B. plurijuga*	Omupapa	SN 1	Fever and headache
	Cyphostemma spp	Kandingila	SN 7	Fever
	G. coleosperma	Omushii	SN 2	Headache, fever cough & malaria
	M. sericea	Omumbanganyana	SN 10	Headache, malaria and fever
	N. oleracea	Namifiilombo	SN 8	Malaria
Ebeneceae	*D. mespiliformis*	Omwandi	SN 6	Fever
Lamiaceae	*A. inflata*	Etwelakuku	SN 3	Headache
Polygonaceae	*O. dregeanum*	Oshikandachefuma	SN 9	Malaria and fever
Rhamnaceae	*Z. mucronata*	Omukekete	SN 5	Fever, diarrhoea, and malaria
Rubiceae	*V. infausta*	Oshimbu	SN 4	Fever, cold, cough and headache

3 Results

3.1 Collection of plants and extracts preparation

Six plants species, namely *A. inflata*, *V. infausta*, *Z. mucronata*, *D. mespiliformis*, *N. oleracea* and *Cyphostemma* spp were collected form Omughete village in Omusati region, while the remaining four: *G. coleosperma*, *M. sericea* and *B. plurijuga* were collected from Enyana Village in Ohagwena region. All plant's taxonomic identities were confirmed by NBRI in Windhoek, Namibia. Half of the plants species belong to the *Fabaceae* family while the rest belong to *Rhamnaceae, Ebeneceae, Lamiaceae, Rubiceae* and *Polygonaceae* each. The rest of

the results are summarised in Table 2.

3.2 Thin layer chromatography analysis

All extracts analysed showed the presence of at least one groups of phytochemical analysed. The shoots of *M. sericea* indicated the presence of all phytochemicals while the whole *A. inflata* plant indicated the presence of terpenoids only. Flavonoids were detected in most leaves although they were absent in roots and bark extracts, alkaloids were detected in barks and leaf extracts. Anthraquinones, coumarins and terpenoids were mostly detected in whole plants and leaf extracts whilst shoots and barks showed the leas. Table 3 gives a summary.

Table 3: Qualitative analysis of major anti- plasmodial classes of compounds of 10 Namibian ethno medicinal plants.

Families		Flavonoids	Alkaloids	Anthraquinones	Terpenoids	Coumarins
Fabaceae	*B. plurijuga*, B	-	+	+	+	-
	B. plurijuga, L	+	-	+	-	-
	B. plurijuga, R	-	+	+	-	-
	Cyphostema spp, WP	+	-	+	+	+
	G. coleosperma, B	-	+	-	+	-
	G. coleosperma, L	+	+	+	-	+
	G. coleosperma, R	-	-	+	-	-
	M. sericea, L	+	-	-	+	-
	M. sericea, S	+	+	+	+	+
	N. oleracea, WP	+	-	+	+	-
Ebeneceae	*D. mespiliformis*, L	-	+	+	+	-
	D. mespiliformis, R	-	+	+	+	+
Lamiaceae	*A. inflata*, WP	-	-	-	+	-
Polygonaceae	*O. dregeanum*, WP	-	-	+	+	+
Rhamnaceae	*Z. mucronata*, R	-	+	-	+	-
	Z. mucronata, L	+	-	+	+	-
Rubiceae	*V. infausta*, S	-	+	-	+	+
	V. infausta, L	+	-	-	+	+
	V. infausta, R	-	+	+	+	+

Key: + = present, - = absent, L= leaves, R= roots, S= shoots, WP= whole plant and B= bark

3.3 Total phenolics and alkaloids quantification

A linear calibration curve of gallic acid and quinine HCL with R^2 of 0.995 and 0.9772 for total phenolics and alkaloids respectively were obtained. The shoots of *V. infausta* indicated the highest source of total phenolic content (444. 07 GA/E μg/ml), followed by *G. coleosperma*'s roots while leaves of *D. mespiliformis* showed the least (99.58 GA/E μg/ml). The leaves of *G. coleosperma* had the highest of total alkaloids (28.12 μg/g extracts), followed by *Cyphostemma* spp whole plant while, the roots of *Z. mucronata* indicated the least estimate

of total alkaloids (0.37 μg/g extracts). Whole plant extracts had the highest total alkaloid content, followed by leaves, roots and lastly bark. The roots had the highest total phenolic content, followed by leaves then roots and lastly bark and shoot. The rest of the results are summarised in the Figure 1 and 2.

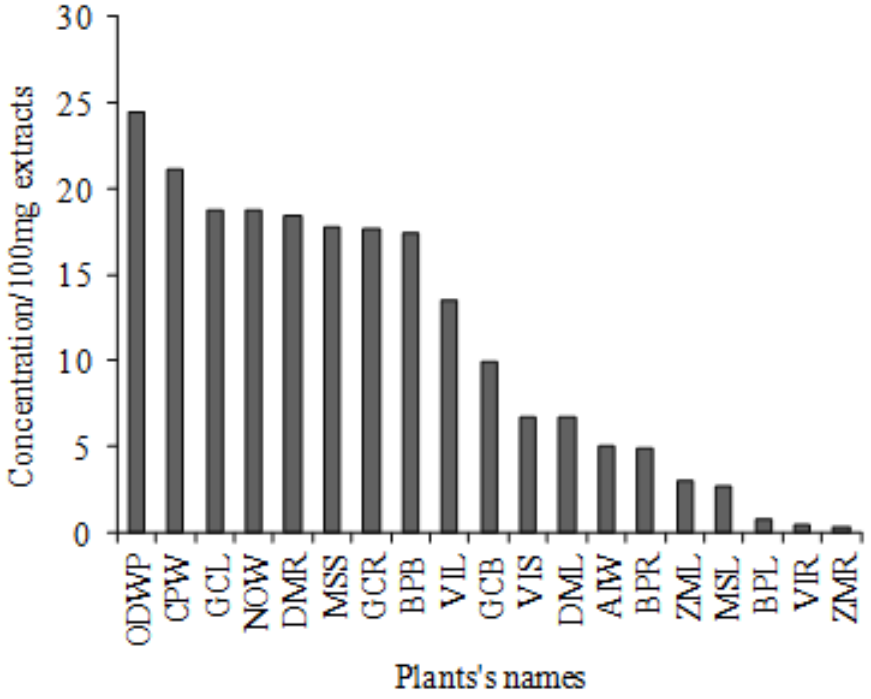

Figure 1: Quantitative estimation of total phenolics content of 10 ethno medicinal plants used to treat febrile illnesses in Northern Namibia. **Abbreviations**: BPB: *Baikiaea plurijuga*, bark. BPL: *Baikiaea plurijuga*, leaves BPR: *Baikiaea plurijuga*, roots, CPW: *Cyphostemma* spp, whole plant, GCB: *Guibourtia coleopsperma*, bark, GCL: *Guibourtia coleopsperma* Leaves, GCR: *Guibourtia coleopsperma*, roots, MSL: *Mundulea sericea*, leaves, MSS: *Mundulea sericea*, shoots. NOW: *Neptunia oleracea*, whole plant, DML: *Diospyros mespiliformis*, leaves, DMR: *Diospyros mespiliformis*, roots AIW: *Acrotome inflata*, whole Plant, ODW: *Oxygonum dregeanum*, whole plant, ZMR: *Ziziphus mucronata*, roots, ZML: *Ziziphus mucronata*, leaves, VIS: *Vangueria infausta*, shoots, VIL: *Vangueria infausta*, leaves. VIR: *Vangueria infausta*, roots

3.4 *In vitro* Anti-plasmodial activity of three ethno medicinal plants used to treat malaria associated symptoms

M. sericea (roots and shoots), *D. mespiliformis* (leaves and roots) and *Cyphostemma* spp, whole plants, were selected for anti-plasmodial bioassay based on the presence of the highest abundance of anti-plasmodial phytochemicals classes of compounds tested and high estimate

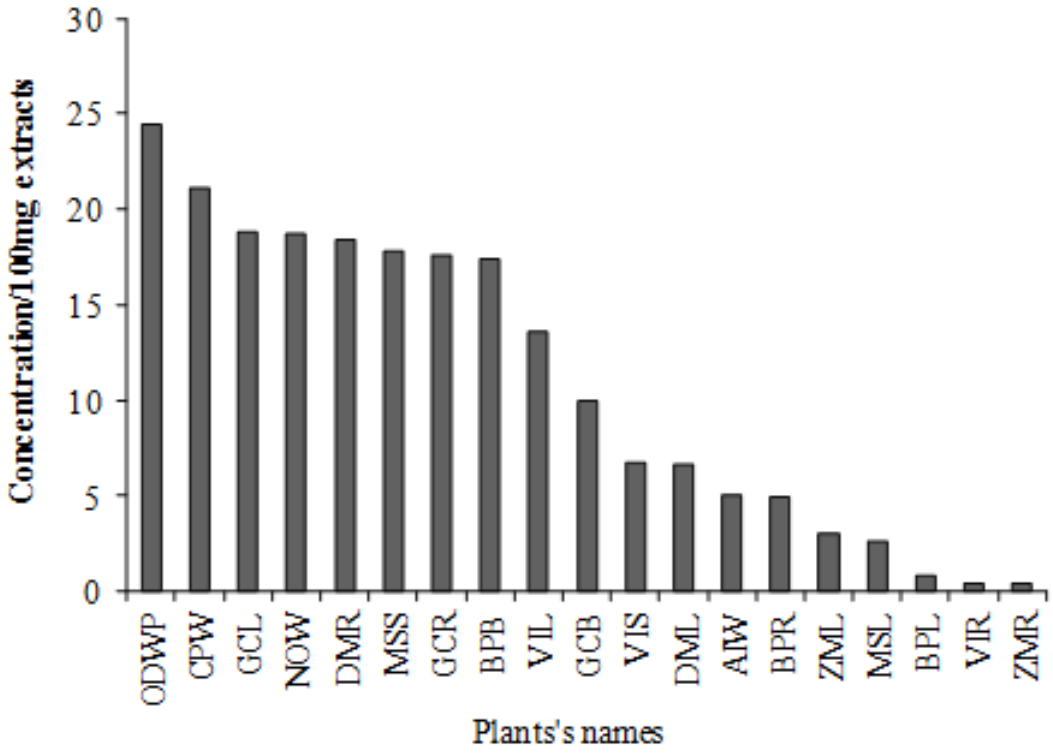

Figure 2: Quantitative estimation of total alkaloids content of 10 ethno medicinal plants used to treat febrile illnesses in Northern Namibia. **Abbreviations**: BPB: *Baikiaea plurijuga*, bark. BPL: *Baikiaea plurijuga*, leaves BPR: *Baikiaea plurijuga*, roots, CPW: *Cyphostemma* spp, whole plant, GCB: *Guibourtia coleopsperma*, bark, GCL: *Guibourtia coleopsperma* Leaves, GCR: *Guibourtia coleopsperma*, roots, MSL: *Mundulea sericea*, leaves, MSS: *Mundulea sericea*, shoots. NOW: *Neptunia oleracea*, whole plant, DML: *Diospyros mespiliformis*, leaves, DMR: *Diospyros mespiliformis*, roots AIW: *Acrotome inflata*, whole Plant, ODW: *Oxygonum dregeanum*, whole plant, ZMR: *Ziziphus mucronata*, roots, ZML: *Ziziphus mucronata*, leaves, VIS: *Vangueria infausta*, shoots, VIL: *Vangueria infausta*, leaves. VIR: *Vangueria infausta*, roots

of total phenolics and alkaloids content (see Figure 1 and 2). The *in vitro* anti plasmodial activity of methanol and aqueous extracts are summarised in the Table 4. In comparison of growth percentages of controls for 48 hours, there was a minimal DMSO effect; therefore, the activity observed can be attributed to the phytochemical content of the plant extracts. According to the set of criteria set by Deharo (2001), plant extracts are considered highly active when its IC_{50} is less than 5 μg/ml, moderately active when its IC_{50} value is between 5 and 10 μg/ml and inactive when its IC_{50} more than 10 μg/ml. The results IC_{50} shown by the table indicate that both aqueous and methanol extracts were active against *P. falciparum* 3D7A strain expect *D. mespiliformis* roots. The methanol extracts were more active than their corresponding aqueous extracts of the same plant parts. The methanol of *D. mespiliformis* roots was the most active with IC_{50} value of 3.179 μg/ml while the methanol leaves extracts of the same plant had the highest IC_{50} of 3.533 μg/ml although considered highly active according to Deharo (2001) classification.

Table 4: IC_{50} values of 3 selected medicinal plants used to treat malaria associated symptoms against *P. falciparum* 3D7A strain.

Plants	Plant parts	Aqueous extracts IC_{50} (μg/ml)	Methanol extracts IC_{50} (μg/ml)
M. sericea	Leaves	9.440	3.352
	Shoots	3.756	3.279
D. mespiliformis	Leaves	7.519	3.523
	roots	18.027	3.179
Cyphostemma spp	Whole plants	6.452	3.276

4 Discussion

The study has evaluated the use of the selected plants by phytochemistry and *in vitro* anti-plasmodial activity in traditional settings in Namibia's malaria endemic region to treat malaria associated symptoms. *M. sericea*'s shoots show the presence of all phytochemicals, and also show the highest total phenolics (274.75 GA/E μg/ml) and total alkaloids content (17.78 μg/g extracts). This can be linked to its effectiveness in treatment of malaria symptoms in traditional setting (Von Koenen 2001). It is noteworthy to mention that deguelin of the flavonoids group of phytochemicals and an anti-cancer natural product, was first isolated from *M. sericea* (Wang et al. 2012; Mehta et al. 2013). *A. inflata*, known as *Etwelakuku* in Oshiwambo language is known for its typical, strong and choking smell and its use as mosquito repellent; in study, this plant indicated the presence of terpenoids only. Terpenoids such as mono, di and sesquiterpenoids contribute to the odour of plants (Bakkali et al. 2008). Terpenoids are also significant to malaria treatment because artemisinin the lead molecule of the artemisinin based drugs, and its derivatives belong to the terpenoids class of compounds (White 2008; Abad et al. 2012). Furthermore, terpenoids were detected in 15 out of 18 extracts as shown in Table 3.

Alkaloids were detected in 10 of the 18 plant extracts analysed. Alkaloids are generally

known as nitrogen containing secondary metabolites with low molecular weight, Evans, (2009) and some members of this group have anti-plasmodial activities (Graziosea et al. 2011; Dolabelaa et al. 2008; Astulla et al. 2008; Oliveira et al. 2009; & Frederich et al. 2008). The presence of coumarins and flavonoids was detected in 8 of 18 extracts analysed, these compounds are part of the phenolic group which are distinguished the presence of one or more hydroxyl group bonded to benzopyrano ring (Jain & Himanshu, 2012). Anti-plasmodial activities of coumarins and flavonoids have been reported as well (Bero & Joelle-Quetin 2010; Bero et al. 2009; Kaur et al. 2009; Batista et al. 2009; Sturm et al. 2009, Voahangy et al. 2008). Most plants indicate the presence of terpenoids (15) and the least were coumarins and flavonoids (8). This represents enormous potential of these plants to contain novel bioactive anti-plasmodial compounds.

The presence of flavonoids in the *V. infausta* leaf extracts and alkaloids in the root extract of *V.infausta* was reported by Mbukwa et al. (2007) and Oluwole et al. (2007), in Botswana and Swaziland, and Nigeria respectively. Furthermore, the presence of terpenoid and alkaloids in both roots and leaves extracts of *D. mespiliformis* is consistent with the findings of Shagal & Kubmarawa (2012), however, the presence of flavonoids in both extract was contradicted by Shagal & Kubmarawa (2012). Variations in phytochemical content of the plants analysed may be influenced by factors such as climatic, geographic, harvesting and storage conditions after and before extractions and analysis, these were not considered in this study (Shagal & Kubmarawa 2012). For the first time, phytochemical profiling of alkaloids, terpenoids, Anthraquinones, flavonoids and coumarins of *A. inflata*, *N. oleracea*, *O. dregeanum* and *Cyphostemma* spp is reported.

Total alkaloid content of the plants ranges from 0.37- 21.21 μg/g of extracts. Alkaloids are naturally rare and confined to certain families of plants such as *Papaveraceae, Rubiceae* etc (Gangwal, 2013). Additionally, alkaloids are difficult to extract (Petruczynik 2012), which may be the rationale for trace amount of total alkaloids estimate detected in some plants. Total phenolics content range from 99. 58 - 336.01 GA/E μg/ml. Phenolics compounds are abundant in many plant's species and ubiquitous in most plants parts (Crozier et al. 2010; D'Archivio et al. 2010 & Cartea et al. 2010). The roots of *G. coleosperma* did not indicate the presence of coumarins or flavonoids but yet it indicated the highest phenolics content of 343.55 GA/E μg/ml, this could be due to other phenolic compounds such as tannins, saponins that were not analysed in this study.

The classifications of good, moderate and inactive *in vitro* anti-plasmodial activity depend on selected criteria. In this study, extracts are considered active when the IC_{50} values of extracts are less than 10 μg/ml and highly active when the IC_{50} is less than 5μg/ml. This is because inhibition of parasite growth at low concentration will indicate selective activity as opposed to high concentration where non-specific activity is usually observed (Clackson et al. 2004). Linear regression analysis was used to calculate IC_{50} values, non-linear regression can also be used to find the curve that best fits the data to calculate the IC_{50} if the statistical software is available. Interestingly, methanol extracts indicated high anti plasmodial activity as opposed to aqueous extracts, which is the main and preferred solvent used in traditional

setting. Possible reasons, water is unable to extract lipophilic phytochemicals that are extracted by methanol and aqueous extracts were not prepared the same way as in traditional setting. Beside, in traditional setting, different plant extracts are mixed into concoction which might enhance their *in vitro* activity. Anti-plasmodial activity observed can be attributed the multi constituent of the extracts as revealed by TLC analysis. The toxicity of DMSO was reduced by diluting to less than 0.5% which is the recommended safe concentration to *P. falciparum* (Cos et al. 2006). To the best of our knowledge this the first time anti-plasmodial activity of the *M. sericea*, *D. mesipiloformis* and *Cyphostemma* spp are reported.

Identification, quantification of anti-plasmodial classes phytochemical and evaluation of the selected plants for in vitro anti-plasmodial is the primary step towards developing the pharmacological potential of these plants. They can provide a viable treatment option to Namibian people who do not use allopathic medicine, and they can provide leads for malaria drug development, especially with the reported resistance of *P. falciparum* to anti- plasmodial drugs.

5 Conclusion

This study used phytochemistry and *in vitro* anti-plasmodial activity to evaluate the use of Namibian medicinal plants to treat malaria associated symptoms and form a basis for development of herbal medicines that can be readily available to people without prompt access to modern anti-malarial agents, or those who choose not to use it.

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Ethical approval

The plants were collected with a research permit granted by the government of the Republic of Namibia, through the Ministry of Environment and Tourism. Phlebotomy was carried with prior consent of donors. A qualified physician conducted phlebotomy.

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