Studies on the in vitro antiplasmodial activity of endemic and native plants from Cabo Verde

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Abstract

Background

Traditional medicinal plants and other remedies are one of the many potential sources of new antimalarial drugs, there being an increasing interest in studying their potential for the treatment of malaria and other illnesses. The Cabo Verde archipelago harbors a rich native plant diversity, most of which are used with medicinal purposes.

Methods

The present study investigated the *in vitro* antiplasmodial activities of four native plants from Cabo Verde. Aqueous and ethanolic extracts of these plants were tested *in vitro* against both chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) *P. falciparum* strains using a SYBR Green detection method, and their cytotoxicity was evaluated in Caco and PLP2 cells by using a sulforhodamine B colorimetric assay.

Results

An ethanolic extract of *Artemisia gorgonum* and an aqueous extract of *Tamarix senegalensis* exhibited high antiplasmodial activity (EC_{50} < 5 µg/mL) without cytotoxicity. Extracts of *Lavandula rotundifolia* and *Sideroxylon marginatum* exhibited moderate activity, with EC_{50} values ranging from 10-30 µg/mL. The *A. gorgonum* ethanolic extract showed activity toward early ring stages, and parasites treated with the *T. senegalensis* aqueous extract progressed to early trophozoite stage but did not develop further to the late trophozoite or schizont stages.

Conclusions

Antimalarial activities as well as the lack of toxicity of the extracts found in the present study support the claim by traditional practitioners for the use of the plants against malaria and suggests their ethnopharmacological usefulness as future antimalarials.

1. Background

Malaria is among the leading health concerns in Africa, having plagued this continent for centuries, causing severe morbidity and mortality (1). *Plasmodium falciparum* is the most prevalent and lethal species infecting humans in the WHO African Region, with 29 African countries in which malaria is endemic being responsible for 96% of malaria cases and deaths in 2020 (2).

The emergence of parasites resistant to currently used antimalarial therapies (3), increasing resistance of vectors to insecticides (4) and the difficulty in developing efficient vaccines and therapies, underpin the urgent need for new means of malaria control. The current situation is especially alarming due to a convergence of threats, ranging from the COVID-19 and Ebola outbreaks, to floods and other humanitarian emergencies that have led to disruptions in national health services structures, impacting on the treatment of several illnesses, including malaria (5). Due to the fragility of the health system in several African

countries and lack of access to physicians, the search for solutions in traditional medicine has increased considerably for diseases such as malaria (6).

Plants have been used throughout the history of humankind as a source of health-inducing remedies, as they constitute a rich reservoir of bioactive secondary metabolites, most of which have been underexplored (7). The first treatment-based malaria control approaches came from traditional herbal medicine: quinine, a component of the bark of the cinchona (quina-quina) tree, and its synthetic derivatives (chloroquine, hydroxychloroquine, amodiaquine, primaquine, and mefloquine); artemisinin from the Chinese medicinal plant *Artemisia annua*, the predecessor of compounds such as artemether, arteether, and sodium artesunate; and atovaquone, which is a synthetic analogue compound (2-alkyl-3-hydroxynaphthoquinone) of lapachol from the *Tabebuia* species (Bignoniaceae) (8). Further studies of medicinal plants throughout the years have led to the discovery of numerous and distinct antimalarial compounds (9–11).

Cabo Verde is a volcanic archipelago located in the Atlantic Ocean with 10 islands, about 600 km off the West African coast (16°0'9"N 24°0'50"W). The archipelago was discovered in the mid-15th century, and most inhabitants of Cabo Verde are of mixed ancestry, combining influences from European and African cultures (12). The position of these islands in the Atlantic Ocean allowed rapid colonization, and several useful plants were introduced mainly by immigrants of different ethnic groups from the West African region (6, 13). The use of plants (e.g., food, medicines and building materials) to satisfy the basic needs of the population is still a common practice in this archipelago (14), and, the use of medicinal plants to treatment various diseases, constitutes an important health resource with significant potential for research and development of new medicines.

In Cabo Verde, malaria has been officially reported since the sixteenth century when the islands were settled (15). As of January 2021, the country completed three consecutive years without local malaria transmission, becoming eligible to apply for the World Health Organization (WHO) certification of malaria elimination for the third time (2). However, surveillance and prevention should remain in place as in Santiago Island, the most populated, there is constant flux of migrants from malaria hyperendemic regions, namely Senegal, São Tomé and Príncipe, Angola, or Guinea- Bissau (16).

As in other countries in Africa, in Cabo Verde traditional medicine has also played a role in combating malaria, although to a lesser extent. According to De Pina et al. (16) only 3% of the population of the country has resorted to traditional remedies as first line treatment upon the appearance of the first malaria symptoms, although there is no reference to the number of people that carried on using traditional remedies while on conventional therapy, reflecting the strength of the public health system regarding malaria treatment.

In this study, four native medicinal plants that are ethnomedically used in the treatment of malaria and malaria-like symptoms (e.g. fevers, shaking chills, and flu-like illness) in Cabo Verde were investigated for their antiplasmodial activities. Crude ethanolic and aqueous extracts from these plants were tested *in vitro* against both chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) *P. falciparum* strains.

2. Methods

2.1. Plant material

All plants (Table 1) were purchased (in March/2021) at the two largest markets (i.e. Assomada's and Praia markets) of Santiago, the largest and more populated island of the archipelago. These plants were identified and later housed in the LISC Herbarium (IICT/ University of Lisbon) and transported to the Laboratory Ferreira Lapa, (Superior Institute of Agronomy), for preparing the extracts.

Table 1
– Plant species studied and their respective traditional uses.

Species (Family)*	Common names	Native status	Medicinal applications**	Parts used	Preparation and administration	Sample Voucher
Artemisia gorgonum Webb (Asteraceae)	Losna, absinto	Endemic	Intestinal & stomach problems, cough and respiratory diseases, fevers, malaria, diarrhea, others	whole plant	Infusion, vaporization and smoked as a cigarette	Silva 2 (LISC Herbarium)
Lavandula rotundifolia Benth. (Lamiaceae)	Aipo, alfazema-brava, alpo-rotcha	Endemic	Pains, stomach and kidney problems, cough and respiratory diseases, fever, malaria, diarrhea, others	leaves, flowers	Infusion, bath and oral administration	Silva 9 (LISC Herbarium)
Sideroxylon marginatum (Decne. ex Webb) Cout. (Sapotaceae)	Marmulano	Endemic	Pains, fever and flu-like illness, rheumatism, bones	leaves, bark	Infusion, maceration, oral and dermal administration	Silva 8 (LISC Herbarium)
Tamarix senegalensis DC. (Tamaricaceae)	Tarrafe, tamargueira	Native	Cough and respiratory diseases, fever and flu-like illness	leaves, fruits	Infusion, bath and oral administration	Silva 10 (LISC Herbarium)
*According to (17)						
***Main references to medicinal uses**: (18–22)						

2.1.1. Plant powder preparation

To enhance the superficial contact and maximize compound content in the solvent media prior to the extraction, room temperature dried plant sample (leaves and stem) were grounded in a lab-scale mixer

(Bimby TM31, Vorwerk, Wuppertal, Germany) and manually sieved with the Analysette 3 Spartan apparatus (Fritsch, Idar-Oberstein, Germany).

2.1.2. Crude Ethanolic Extracts

Biomass of each extract (0.5 g) were suspended in 10 mL of EtOH (96%). The suspension was mixed thoroughly for 30 min at room temperature. After extraction, the supernatants were vacuum filtered with a qualitative paper filter (filter paper VWR 2–3 µm). The solvent was removed by reduced pressure using a rotary evaporator (BUCHI Rotavapor R-114, Lausanne, Switzerland) under partial vacuum at 40°C. The dried extracts were weighted and stored at 4ºC until further use.

2.1.3. Preparation of the aqueous extracts

An aqueous extract was prepared by infusion, pouring 10 ml of sterile distilled water at 95ºC water at the prepared powdered plant and let it rest for 30 min, manually shaking the mixture at each 10 mins and then cooling to room temperature. The aqueous extracts were subsequently filtered using Millipore filters (Millipore 0.2 mm) to remove particulate material. The resulting infusion was lyophilized to ensure accurate dosing and a long shelf-life of the freshly prepared aqueous extract. For this, the infusion was frozen at − 20.0°C and dried in a Heto Dry Winner freeze dryer (Denmark) at residual pressure 0.1 − 0.03 mbar and at room temperature for 22–24 h (moisture 5%).

2.1.4. Determination of Percentage Yield (%)

The percentage yield was determined by subtracting the weight of the flask containing the dry extract (W2) from the weight of the flask with dissolved extracts (W1), and dividing that value for of weight of dried plant material (W0) using the following equation:

$$Yield(\%) = \frac{(W2 - W1)}{W0} 100$$

Extract/fraction was calculated as the average of three replicates.

2.2. *Plasmodium* Culture

Chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) strains were cultured in RPMI 1640 medium supplemented with 0.05 mg/mL gentamycin, 38.4 mM HEPES, 0.2% sodium bicarbonate, and 5% Albumax II, as previously described in a standardized protocol (23). Then, erythrocytes were added to the culture to obtain a 5% hematocrit and incubated at 37°C under 5% CO_2 atmosphere, with daily exchange of medium. The parasitemia was monitored daily in smears stained with Giemsa. Synchronic cultures in the ring stage were obtained by two consecutive treatments at 48 h intervals with a 5% solution of D-sorbitol (24).

2.2.1. Antiplasmodial *in vitro* assay

Extracts were added in serial dilutions (100 − 0.05 µg/mL) into clear bottom 96-well plates (Costar #3904). *P. falciparum* cultures were added at a 2% hematocrit with a 0.5% parasitemia and incubated for 72 h at 37°C. Plates were frozen at -20°C overnight. Lysis buffer (20 mM Tris-HCl, 5 mM EDTA, 0.008% saponin, 0.08%

Triton X-100, and 0.4 µl/ml SYBR Green I dye (Invitrogen #S7585) was added to a new black 96-well plate. After the addition of 100 µL of lysate, the plates were incubated in the dark for 60 min and read at 490 nm excitation and 540 nm emission. EC_{50} was estimated by nonlinear regression analysis using Prism GraphPad software by plotting Log dosing vs growth inhibition (expressed as percentage relative to the drug-free control) (25)

2.2.2. Morphological changes in parasites by microscopy

Highly synchronous 3D7 parasites were obtained by Percoll treatment and subsequent sorbitol treatment (after 2h of incubation) to remove any left multinucleated schizonts. The remaining rings (0–2 hours) were then maintained at 2% haematocrit and morphological changes of treated parasites were observed at different time points (0, 10, 20, 30, 40, 45, and 60 hour).

2.3. Cytotoxic activity

The cytotoxic activity of the extracts was assessed as previously described by (26) using the sulforhodamine B (Sigma-Aldrich, St. Louis, MO, USA) colorimetric assay against Caco cells (colorectal adenocarcinoma) (Leibniz-Institut DSMZ). A non-tumor cell line (PLP2 - primary culture of pig liver culture) was also tested. Ellipticine was used as a positive control. The results were expressed as GI_{50} values (µg/mL), which translate the extract concentration responsible for 50% of inhibition of cell proliferation.

2.4. Hemolysis assay

In vitro hemolysis assay was evaluated as described elsewhere (27). Briefly, freshly RBCs were incubated with compounds and after 24 h the supernatants were collected, and the absorbance was measured at 540 nm. The hemolytic rate was determined in relation to the positive control (0.1% Triton-100), which was considered 100% of hemolysis.

3. Results

The native plants chosen for this study were obtained from informal discussions with local traditional healers, indigenes, and traditional medicine's Assomada's market traders. The referred plants belong to different families and are used in treating different ailments such as fever, malaria, stomach aches, and infections.

3.1. Percentage Yield of crude plant material

Table 2 summarizes the concentration and yield of the plant extracts tested in the present study. *A. gorgonum* (20% for the H_2O extract and 7.3% for the EtOH extract), had the highest extraction yield, both for aqueous and ethanolic extracts, followed by *S. marginatum* (18% for the H_2O extract and 5.7% the EtOH extract) and *L. rotundifolia* (18% for the H_2O extract and 4.9% for the EtOH extract).

Table 2
Percentage yield and concentration of aqueous and ethanolic plant extracts used.

Scientific name	Extraction yield (%) H_2O	Concentration (µg/mL) H_2O	Extraction yield (%) EtOH	Concentration (µg/mL) EtOH
Artemisia gorgonum	20	100.3	7.3	36.5
Lavandula rotundifolia	18	90.1	4.9	24.5
Sideroxylon marginatum	18	90.3	5.7	28.5
Tamarix senegalensis	8	40.4	2.8	14.0

3.2. Antiplasmodial activity

The antiplasmodial activity of four medicinal plants commonly used in Cabo Verde was evaluated *in vitro* against two *P. falciparum* strains: the drug-sensitive 3D7 and the multidrug-resistant Dd2 (which is resistant to several antimalarials, including chloroquine, mefloquine, quinine, pyrimethamine and sulfadoxine) and the results are shown in Table 3. EC_{50} values of ranged from 1.7 to ≥ 100 µg/mL.

Table 3
– EC_{50} values of studied plant extracts against *P. falciparum.*

	3D7		Dd2	
	EC_{50} (µg/mL) H_2O	EC_{50} (µg/mL) EtOH	EC_{50} (µg/mL) H_2O	EC_{50} (µg/mL) EtOH
L. rotundifolia	95.4 ± 13.2*	26.3 ± 3.2	83.7 ± 19.6*	32.0 ± 7.8
T. senegalensis	4.7 ± 0.6*	11.4 ± 2.1	5.4 ± 1.2*	13.8 ± 3.3
A. gorgonum	49.1 ± 12.3*	1.7 ± 0.5	52.1 ± 11.3*	3.3 ± 0.6
S. marginatum	48.6 ± 5.6*	25.6 ± 2.2	46.0 ± 8.7*	32.0 ± 5.8

Values are presented as mean ± standard deviation representative of three independent experiments. Chloroquine was used as positive control, with EC_{50} = 0.008 and 0.13 µg/mL against 3D7 and Dd2, respectively. * $p < 0.05$ by t-test (aqueous x ethanolic extract).

Of the tested extracts, two of the plants exhibited high antiplasmodial activity ($EC_{50} \leq 5$ µg/mL), namely the *A. gorgonum* ethanolic extract and the *T. senegalensis* aqueous extracts. Ethanolic extracts of *T. senegalensis*, *L. rotundifolia* and *S. marginatum* showed moderate antiplasmodial activity ($EC_{50} > 5$ to ≤ 30 µg/mL) against the 3D7 strain. Overall, ethanolic extracts were most potent in comparison to aqueous extract, with exception of *T. senegalensis*. Interestingly, there were no significant differences in the activity

between the sensitive (3D7) and multidrug-resistant (Dd2) strains, suggesting no cross-resistance between the extracts and antimalarials such as chloroquine, pyrimethamine, and cycloguanil.

3.3. *In vitro* effect of *A. gorgonum* and *T. senegalensis* on *P. falciparum* morphology

To characterize the morphological changes in response to the two most active extracts (*A. gorgonum* ethanolic extract and *T. senegalensis* aqueous extract), synchronized *P. falciparum* 3D7 parasites were exposed to a concentration 5-fold greater than the respective extract EC_{50} value. The *A. gorgonum* ethanolic extract showed activity toward early rings, since morphological changes could be observed 10 h after treatment. No viable parasites were observed in the following time points. Parasites treated at ring stages with *T. senegalensis* aqueous extract progressed to early trophozoite stage (20–30 h), but did not progress further to the late trophozoite or schizont stages. (Fig. 1).

3.4. Hemolysis assay and Cytotoxicity

To confirm that the antiplasmodial activity of the extracts was not due to erythrocyte lysis, we performed a hemolysis assay. All plant extracts were non-haemolytic at 50 µg/mL concentration (Fig. 2**)**. Furthermore, we evaluated the cytotoxicity of the two most potent plants against the parasite (*A. gorgonum* and *T. senegalensis*) using two cell lineages. Only the ethanolic extract of *A. gorgonum* showed moderate cytotoxicity against Caco cells (GI_{50} = 17.3 µg/mL), while no toxicity towards PLP2 cells was observed (Table 4).

Table 4
Cytotoxicity and anti-inflammatory activities of the ethanolic and infusion extracts

Cell	Solvent of extraction	T. senegalensis (GI_{50} (µg/mL)	SI	A. gorgonum (GI_{50} (µg/mL)	SI
Caco	Ethanolic	125 ± 4	11	17.3 ± 0.2	10
	Aqueous	> 400	> 85	181 ± 10	3.7
PLP2	Ethanolic	178 ± 3	15	> 400	> 235
	Aqueous	> 400	> 85	> 400	> 8

SI: selectivity index calculated between GI_{50} on cells and EC_{50} in 3D7 strain (mean + SD, n = 3).

4. Discussion

In the present work we follow traditional methods of preparation of medicinal remedies originally aimed at treating several diseases, including malaria. Infusion is the most common method of consuming these traditional remedies; however there are some traditional practices that include the infusion of these same medicinal herbs in “grogu” or “grog”, a highly alcoholic beverage of artisanal production (22). We aimed at

verifying the extracts' therapeutic potential, comparing two different solvents with the same time of contact with the plant powder. Regarding the extraction yield, factors such as extraction method, solvent used, contact time with extraction solvent and temperature, will largely influence the extraction yield. Water, being the most polar solvent, was able to carry a higher quantity of phytochemicals than ethanol. According with several studies, solvent polarity significantly affects the extract yield, so highly polar solvents resulting in high extract yield as compared to least polar ones (28, 29). Water and ethanol are often recommended for extract preparation due to their differences in polarity and their lower probability to interfere with the phytochemical structure and properties (30). Solvent polarity is also known to affect the quality of crude extracts secondary metabolites, and biological activities (31).

The results of the present study demonstrated that both aqueous and ethanolic extracts of some plants currently used in traditional Cabo Verde medicine exhibit antiplasmodial efficacy, some of them being classified as highly active ($EC_{50} \leq 5$ µg/mL) (according to WHO and previous works) (32, 33) against *P. falciparum*. Amongst the tested plants, the one that presented the higher activity was, unsurprisingly, *A. gorgonum*. *A. gorgonum* belongs to the list of Cabo Verde's endemic plants and, although this is the first study testing its crude aqueous and ethanolic extracts against malaria, its volatile oil had been tested previously *in vitro* for its antimalarial properties, having presented an EC_{50} of 5.2 µg/mL (34). Later, approximately 14 compounds were isolated from *A. gorgonum* leaves and flowers and tested separately, and three phytochemicals, ridentin, sesamin and artemetin presented promising activity against *P. falciparum* with an EC_{50} between 3.3 and 3.8 µg/mL. (34, 35). Some metabolites isolated from this species have also been tested regarding their anti-tumoral ability with promising results (19), and its hepatoprotective potential as well (36). *Artemisia* genera belongs to the Asteraceae family and 500 species have been reported, the majority of which are found in the temperate areas of Asia, Europe, and North America. Its use in traditional herbal medicine in several cultures is widespread, with many of its ethnopharmaceutical properties having been validated both by traditional practitioners as by conventional scientific knowledge. The Chinese *A. annua* was the species from which artemisinin, an endoperoxide sesquiterpene lactone, was firstly isolated, and now ACT (Artemisinin Combined Therapy) is first-line malaria treatment. In fact, the identification of artemisinin represented a breakthrough in 20th century tropical medicine, contributing to saving millions of lives in South China, Southeast Asia, Africa, and South America. The promising EC_{50} of the crude ethanolic extract of *A. gorgonum* reinforces the fact that this species can be a potential starting point for the development of future antimalarial drugs.

The antiplasmodial activity of the plant extracts will sometimes be dependent on the concentration of some active antimalarial secondary metabolite/s. Gedunin, for instance, a highly active anti-plasmodial metabolite (EC_{50} of 0.02 µg/mL) isolated from the leaves of *Azadirachta indica*, presents low concentrations in the plant, resulting thus in moderate activity of its extract (37, 38). On the other hand, the ethanolic plant extract of *A. Afra* displays an EC_{50} of 2.66 µg/mL (39), which is more potent than its isolated flavonoids acacetin, genkwanin and 7-methoxyacacetin whose EC_{50} values ranged from 4.3 µg/mL to 12.6 µg/mL (40), suggesting that its secondary metabolites may act synergistically.

The present study also showed for the first time the antiplasmodial potential of *Tamarix senegalensis* infusion with EC_{50}s of approximately 5 µg/mL against both sensitive and multidrug-resistant *P. falciparum* strains. The native range of this species extends from Cabo Verde to the Arabian Peninsula and Iran to Pakistan, and although no study has been carried out to our knowledge with this species, ethnopharmacological studies have been developed with some other *Tamarix* spp (41–43). Persian traditional medicinal plants from this species are believed to have cleansing effects on internal organs, especially on the liver and spleen, which are known to be affected in severe malaria patients (44). Calabrian people, in the southern region of Italy, used this plant both prophylactically and therapeutically to heal malaria, between the end of the 19th century and the 20th century. Additionally, some species from the same genera have been shown to present antiparasitic activities, such as *T. aphylla* (45) and *T. ramosissima* (46), both being effective against *Leishmania*.

5. Conclusions

This work provides evidence to support the ethnopharmacological use of *A. gorgonum* and *T. senegalensis* ethanolic and aqueous extracts in the management of malaria. Further studies to identify and isolate the active compounds from these extracts will aid in the development of new antimalarials.

Declarations

Ethics approval and consent to participate

This work was developed under a collaboration protocol between Cape Verdean institutions (Universidade de Cabo Verde (Uni-CV), Instituto Nacional de Investigação e Desenvolvimento Agrário (INIDA) and Portuguese (Instituto Superior de Agronomia (ISA) and Faculdade de Ciências e Tecnologia of the Universidade dos Açores) under the project "Climatic changes and plant genetic resources: the overlooked potential of Cape Verde's endemic flora" CVAgrobiodiversity/333111699, funded by the "Aga Khan Development Network" (AKDN) and the "Fundação para a Ciência e Tecnologia" (FCT). The team includes senior researchers and post-graduate students from Cape Verde (APE, IG) and Portuguese researchers (MM, MMR). The study is strictly of a scientific nature, not including any development component, and the necessary licenses were obtained under the collaboration protocol above mentioned.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Conceptualization, PC, MMR; methodology, AE; GC; FM; MML PC; MMR; Laboratory analyses, AE; GC; FM; MML PC; field surveys, AE, IG, MMR; investigation, AE, GC, MMR, PC; supervising, PC, MMR; writing—original draft preparation, AE, GC; writing—review and editing, PC, MM, MMR. All authors have reviewed and approved the submitted version of this manuscript.

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References

1. Badmos AO, Alaran AJ, Adebisi YA, Bouaddi O, Onibon Z, Dada A, et al. What sub-Saharan African countries can learn from malaria elimination in China. Trop Med Health [Internet]. 2021 Dec 24;49(1):86. Available from: https://tropmedhealth.biomedcentral.com/articles/10.1186/s41182-021-00379-z
2. WHO. Word Malaria Report 2021. Word Malaria report Geneva: World Health Organization. (2021). Licence: CC. 2021. 2013–2015 p.
3. Blasco B, Leroy D, Fidock DA. Antimalarial drug resistance: linking Plasmodium falciparum parasite biology to the clinic. Nat Med [Internet]. 2017 Aug 4;23(8):917–28. Available from: http://www.nature.com/articles/nm.4381
4. Riveron JM, Tchouakui M, Mugenzi L, Menze BD, Chiang M-C, Wondji CS. Insecticide Resistance in Malaria Vectors: An Update at a Global Scale. In: Towards Malaria Elimination - A Leap Forward [Internet]. InTech; 2018. Available from: http://www.intechopen.com/books/towards-malaria-elimination-a-leap-forward/insecticide-resistance-in-malaria-vectors-an-update-at-a-global-scale
5. Gebremeskel AT, Otu A, Abimbola S, Yaya S. Building resilient health systems in Africa beyond the COVID-19 pandemic response. BMJ Glob Heal [Internet]. 2021 Jun 28;6(6):e006108. Available from: https://gh.bmj.com/lookup/doi/10.1136/bmjgh-2021-006108
6. Catarino L, Havik PJ, Romeiras MM. Medicinal plants of Guinea-Bissau: Therapeutic applications, ethnic diversity and knowledge transfer. J Ethnopharmacol [Internet]. 2016 May;183:71–94. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378874116300757
7. Seca AML, Pinto DCGA. Biological Potential and Medical Use of Secondary Metabolites. Medicines [Internet]. 2019 Jun 12;6(2):66. Available from: https://www.mdpi.com/2305-6320/6/2/66
8. Ravelo Á g., Estévez-braun A, Pérez-sacau E. The chemistry and biology of lapachol and related natural products α and β-lapachones. In 2003. p. 719–60. Available from:

https://linkinghub.elsevier.com/retrieve/pii/S1572599503800170

9. Pan W-H, Xu X-Y, Shi N, Tsang S, Zhang H-J. Antimalarial Activity of Plant Metabolites. Int J Mol Sci [Internet]. 2018 May 6;19(5):1382. Available from: http://www.mdpi.com/1422-0067/19/5/1382
10. Tajbakhsh E, Kwenti TE, Kheyri P, Nezaratizade S, Lindsay DS, Khamesipour F. Antiplasmodial, antimalarial activities and toxicity of African medicinal plants: a systematic review of literature. Malar J [Internet]. 2021 Dec 25;20(1):349. Available from: https://malariajournal.biomedcentral.com/articles/10.1186/s12936-021-03866-0
11. Mohammadi S, Jafari B, Asgharian P, Martorell M, Sharifi-Rad J. Medicinal plants used in the treatment of Malaria: A key emphasis to Artemisia, Cinchona, Cryptolepis, and Tabebuia genera. Phyther Res [Internet]. 2020 Jul 5;34(7):1556–69. Available from: https://onlinelibrary.wiley.com/doi/10.1002/ptr.6628
12. Romeiras MM, Duarte MC, Santos-Guerra A, Carine M, Francisco-Ortega J. Botanical exploration of the Cape Verde Islands: From the pre-Linnaean records and collections to late 18th century floristic accounts and expeditions. Taxon [Internet]. 2014 Jun 30;63(3):625–40. Available from: http://doi.wiley.com/10.12705/633.37
13. Romeiras MM, Catarino L, Torrão MM, Duarte MC. Diversity and origin of medicinal exotic flora in Cape Verde Islands. Plant Ecol Evol [Internet]. 2011 Jun 15;144(2):214–25. Available from: https://www.ingentaconnect.com/contentone/botbel/plecevo/2011/00000144/00000002/art00006
14. Duarte MC, Gomes I, Catarino S, Brilhante M, Gomes S, Rendall A, et al. Diversity of Useful Plants in Cabo Verde Islands: A Biogeographic and Conservation Perspective. Plants [Internet]. 2022 May 15;11(10):1313. Available from: https://www.mdpi.com/2223-7747/11/10/1313
15. DePina AJ, Stresman G, Barros HSB, Moreira AL, Dia AK, Furtado UD, et al. Updates on malaria epidemiology and profile in Cabo Verde from 2010 to 2019: the goal of elimination. Malar J [Internet]. 2020 Dec 23;19(1):380. Available from: https://malariajournal.biomedcentral.com/articles/10.1186/s12936-020-03455-7
16. DePina AJ, Dia AK, de Ascenção Soares Martins A, Ferreira MC, Moreira AL, Leal SV, et al. Knowledge, attitudes and practices about malaria in Cabo Verde: a country in the pre-elimination context. BMC Public Health [Internet]. 2019 Dec 1;19(1):850. Available from: https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-019-7130-5
17. POWO. Plants of the World Online. Facilited by the Royal Botanic Gardens, Kew [Internet]. 2022 [cited 2021 Apr 21]. Available from: http://www.plantsoftheworldonline.org
18. Gonçalves ML. Flora de Cabo Verde. Plasntas vasculares. 32. Cucurbitaceae. 1996. 1–21 p.
19. Martins A, Mignon R, Bastos M, Batista D, Neng NR, Nogueira JMF, et al. In vitro Antitumoral Activity of Compounds Isolated from Artemisia gorgonum Webb. Phyther Res [Internet]. 2014 Sep;28(9):1329–34. Available from: https://onlinelibrary.wiley.com/doi/10.1002/ptr.5133
20. Romeiras MM, Catarino S, Filipe AF, Magalhães MF, Duarte MC, Beja P. Species Conservation Assessments in Oceanic Islands: the Consequences of Precautionary Versus Evidentiary Attitudes. Conserv Lett [Internet]. 2016 Jul;9(4):275–80. Available from: https://onlinelibrary.wiley.com/doi/10.1111/conl.12212

21. Guil-Guerrero JL, López-Martínez JC, Navarro-Juárez R, Gómez-Mercado F. Gamma-linolenic acid from Cape Verdian Boraginaceae. Nat Prod Res [Internet]. 2006 Jan;20(1):9–12. Available from: http://www.tandfonline.com/doi/abs/10.1080/14786410500166402
22. Gomes A, Vasconcelos T, Almedida M de. Plantas na medicina tropical de Cabo Verde. 2008;1–13.
23. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science [Internet]. 1976 Aug 20;193(4254):673–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/781840
24. Lambros C, Vanderberg JP. Synchronization of Plasmodium falciparum erythrocytic stages in culture. J Parasitol [Internet]. 1979 Jun;65(3):418–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/383936
25. Hartwig CL, Ahmed AOA, Cooper RA, Stedman TT. SYBR Green I®-based parasite growth inhibition assay for measurement of antimalarial drug susceptibility in Plasmodium falciparum. In: Methods in Malaria Research. 2013. p. 122–9.
26. Spréa RM, Fernandes Â, Calhelha RC, Pereira C, Pires TCSP, Alves MJ, et al. Chemical and bioactive characterization of the aromatic plant Levisticum officinale W.D.J. Koch: a comprehensive study. Food Funct [Internet]. 2020;11(2):1292–303. Available from: http://xlink.rsc.org/?DOI=C9FO02841B
27. de Sena Pereira VS, Silva de Oliveira CB, Fumagalli F, da Silva Emery F, da Silva NB, de Andrade-Neto VF. Cytotoxicity, hemolysis and in vivo acute toxicity of 2-hydroxy-3-anilino-1,4-naphthoquinone derivatives. Toxicol Reports [Internet]. 2016;3:756–62. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2214750016300749
28. Razak MFBA, Yong PK, Shah ZM, Abdullah LC, Yee SS, Yaw ITCS. The Effects of Varying Solvent Polarity on Extraction Yield of Orthosiphon stamineus Leaves. J Appl Sci [Internet]. 2012 May 15;12(11):1207–10. Available from: https://www.scialert.net/abstract/?doi=jas.2012.1207.1210
29. Nawaz H, Shad MA, Rehman N, Andaleeb H, Ullah N. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds. Brazilian J Pharm Sci [Internet]. 2020;56. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1984-82502020000100501&tlng=en
30. Abubakar A, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci [Internet]. 2020;12(1):1. Available from: http://www.jpbsonline.org/text.asp?2020/12/1/1/277199
31. Wakeel A, Jan SA, Ullah I, Shinwari ZK, Xu M. Solvent polarity mediates phytochemical yield and antioxidant capacity of Isatis tinctoria. PeerJ [Internet]. 2019 Oct 9;7:e7857. Available from: https://peerj.com/articles/7857
32. Pink R, Hudson A, Mouriès M-A, Bendig M. Opportunities and Challenges in Antiparasitic Drug Discovery. Nat Rev Drug Discov [Internet]. 2005 Sep 1;4(9):727–40. Available from: https://www.nature.com/articles/nrd1824
33. Kumari P, Sahal D, Jain SK, Chauhan VS. Bioactivity Guided Fractionation of Leaves Extract of Nyctanthes arbor tristis (Harshringar) against P falciparum. Costa FTM, editor. PLoS One [Internet]. 2012 Dec 26;7(12):e51714. Available from: https://dx.plos.org/10.1371/journal.pone.0051714

34. Ortet R, Prado S, Regalado EL, Valeriote FA, Media J, Mendiola J, et al. Furfuran lignans and a flavone from Artemisia gorgonum Webb and their in vitro activity against Plasmodium falciparum. J Ethnopharmacol [Internet]. 2011 Nov;138(2):637–40. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378874111007100
35. Rocha e Silva LF, da Silva Pinto AC, Pohlit AM, Quignard ELJ, Vieira PPR, Tadei WP, et al. In Vivo and In Vitro Antimalarial Activity of 4-Nerolidylcatechol. Phyther Res [Internet]. 2011 Aug;25(8):1181–8. Available from: https://onlinelibrary.wiley.com/doi/10.1002/ptr.3424
36. Rodrigues N, Almeida A, Silva H, Pinto D, Seca A, Pereira ML. Potencial Anti-inflamatory Effects of Artemisia Gorgonum on Rat Liver Injury Induced by CCl 4. Microsc Microanal [Internet]. 2016 Mar 14;22(S4):26–7. Available from: https://www.cambridge.org/core/product/identifier/S1431927616000325/type/journal_article
37. MacKinnon S, Durst T, Arnason JT, Angerhofer C, Pezzuto J, Sanchez-Vindas PE, et al. Antimalarial Activity of Tropical Meliaceae Extracts and Gedunin Derivatives. J Nat Prod [Internet]. 1997 Apr 1;60(4):336–41. Available from: https://pubs.acs.org/doi/10.1021/np9605394
38. Braga TM, Rocha L, Chung TY, Oliveira RF, Pinho C, Oliveira AI, et al. Biological Activities of Gedunin—A Limonoid from the Meliaceae Family. Molecules [Internet]. 2020 Jan 23;25(3):493. Available from: https://www.mdpi.com/1420-3049/25/3/493
39. Kane NF, Kyama MC, Nganga JK, Hassanali A, Diallo M, Kimani FT. Comparison of phytochemical profiles and antimalarial activities of Artemisia afra plant collected from five countries in Africa. South African J Bot [Internet]. 2019 Sep;125:126–33. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0254629919309020
40. Kraft C, Jenett-Siems K, Siems K, Jakupovic J, Mavi S, Bienzle U, et al. In vitro antiplasmodial evaluation of medicinal plants from Zimbabwe. Phyther Res [Internet]. 2003 Feb;17(2):123–8. Available from: https://onlinelibrary.wiley.com/doi/10.1002/ptr.1066
41. Alrumman SA. Phytochemical and Antimicrobial Properties of Tamarix aphylla L. Leaves Growing Naturally in the Abha Region, Saudi Arabia. Arab J Sci Eng [Internet]. 2016 Jun 24;41(6):2123–9. Available from: http://link.springer.com/10.1007/s13369-015-1900-x
42. A. Al-Judaibi A. Tamarix arabica and Salvadora persica as antibacterial agents. AIMS Microbiol [Internet]. 2020;6(2):121–43. Available from: http://www.aimspress.com/article/10.3934/microbiol.2020008
43. Bahramsoltani R, Kalkhorani M, Abbas Zaidi SM, Farzaei MH, Rahimi R. The genus Tamarix: Traditional uses, phytochemistry, and pharmacology. J Ethnopharmacol [Internet]. 2020 Jan;246:112245. Available from: https://linkinghub.elsevier.com/retrieve/pii/S037887411932567X
44. Milner DA. Malaria Pathogenesis. Cold Spring Harb Perspect Med [Internet]. 2018 Jan;8(1):a025569. Available from: http://perspectivesinmedicine.cshlp.org/lookup/doi/10.1101/cshperspect.a025569
45. Iqbal J, Zaib S, Farooq U, Khan A, Bibi I, Suleman S. Antioxidant, Antimicrobial, and Free Radical Scavenging Potential of Aerial Parts of Periploca aphylla and Ricinus communis. ISRN Pharmacol [Internet]. 2012 Jul 11;2012:1–6. Available from: https://www.hindawi.com/journals/isrn/2012/563267/

46. Akya A, Chegene Lorestani R, Zhaleh H, Nemati Zargaran F, Ghadiri K, Rostamian M. Effect of Vigna radiata, Tamarix ramosissima and Carthamus lanatus extracts on Leishmania major and Leishmania tropica: An in vitro study. Chinese Herb Med [Internet]. 2020 Apr;12(2):171–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1674638420300174

Figures

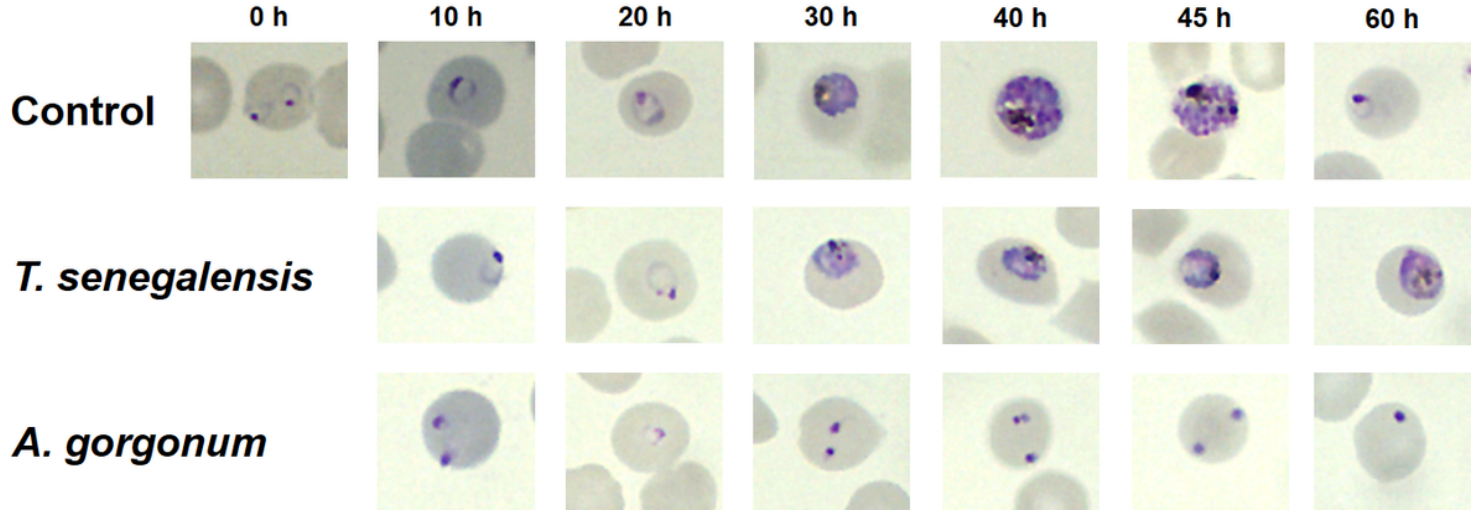

Figure 1

Treatment with *T. senegalensis* aqueous extract prevents the trophozoite development while treatment with *A. gorgonum* acts against early rings. Smears of synchronized 3D7 parasites treated with 5-fold EC_{50} concentration were stained with Giemsa. Untreated control is represented in the first line.

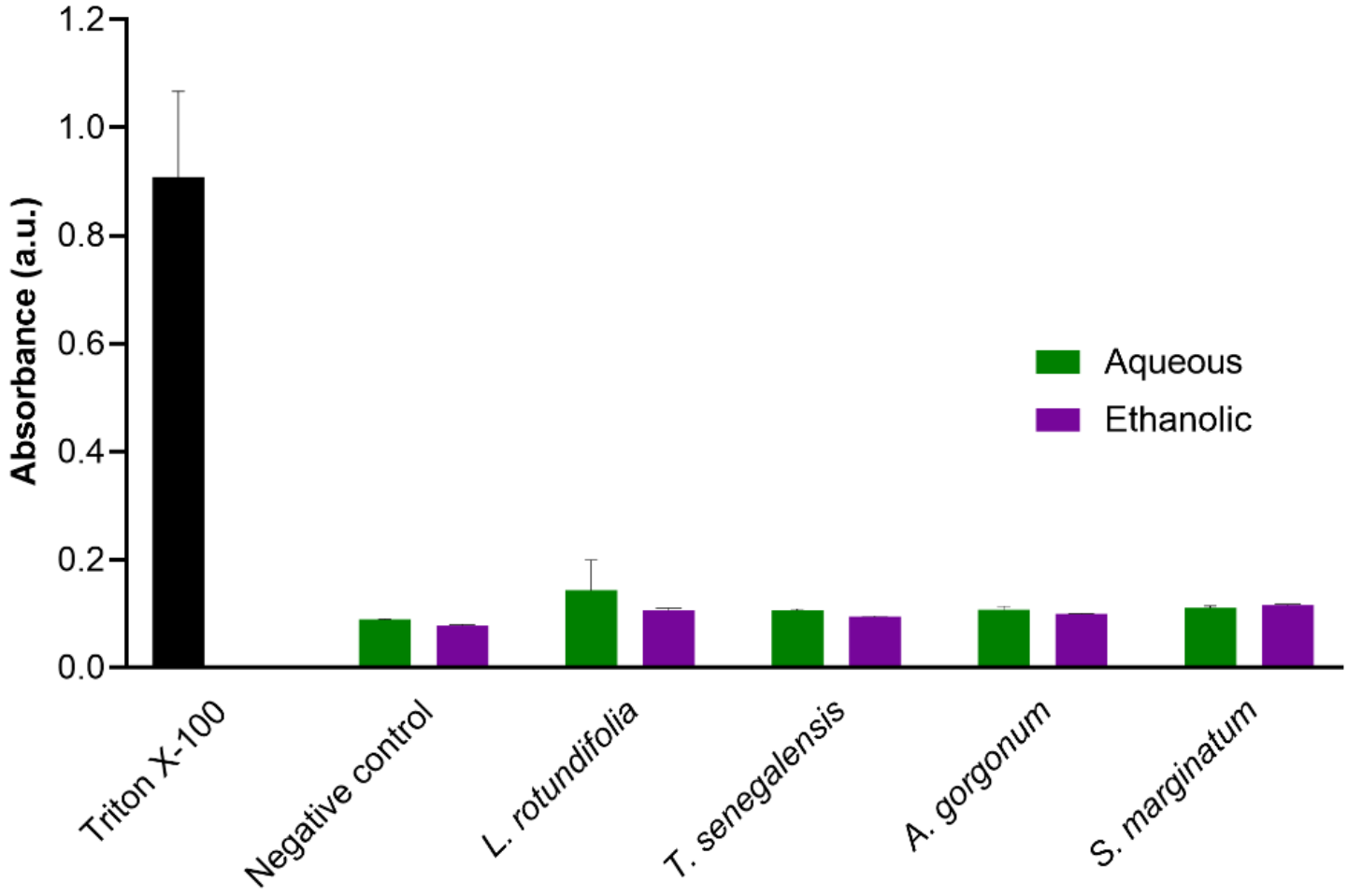

Figure 2

Hemolysis was measured in the presence of extracts at 50 µg/mL after 24h of incubation. Triton X-100 as used a positive control. The data are derived from two independent experiments.