Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.006723

CC BY

The effect of *Eucalyptus camaldulensis* leaf extracts from different environmental harvesting locations on *Plasmodium chabaudi*-induced malaria outcome

Mohamed Abdelmonem DKHIL[1,2*], Hossam Mohammed Abdullah ALJAWDAH[3], Rewaida ABDEL-GABER[3], Felwa Abdullah THAGFAN[4], Denis DELIC[5], Saleh AL-QURAISHY[3]

Abstract

In every country in the globe, malaria is still regarded as a health risk. In terms of therapeutic medicines against parasites, medicinal plants are a promising source, although the types and concentrations of their active compounds can vary depending on the environment. In this study, the effect of *Eucalyptus camaldulensis* leaf extracts (ECE) collected from two different habitats (sandy and muddy) on the antioxidant and antimalarial activity was examined. Phytochemical analysis of ECE from muddy (ECEM) and sandy (ECES) habitats were compared. ECEM contained 13 expected compounds while ECES contained only 10 compounds. This was evidenced through infrared spectroscopy. Also, ECEM contained more phenolics and flavonoids than ECES as well as processes higher antioxidant activity that reached 55%. *Plasmodium chabaudi*-infected mice were treated with ECEM and ECES and the parasitemia was compared. ECEM could significantly suppress the parasitemia by approximately 84% and also was able to decrease the spleen index better than EXCS. Moreover, ECEM was better than ECES in ameliorating the induced decrease in erythrocyte number and hemoglobin content in mice infected with *P. chabaudi*. It is possible to use *E. camaldulensis*, which grows in muddy environments, as an antimalarial drug with the largest efficacy gradients.

Keywords: *Eucalyptus camaldulensis*; malaria; muddy; sandy; habitat.

Practical Application: Effect of harvesting locations of *Eucalyptus camaldulensis* on malaria outcome.

1 Introduction

Natural products' structural diversity, as well as their ability to interact with therapeutic targets, warrant their investigation in the search for new drugs. The majority of the available drugs are of naturally derived (Newman & Cragg 2016). Since ancient times, medicinal plants have been used to treat malaria, and they are promising sources for identifying candidates for novel anti-malarial agents (Dkhil et al., 2021; Aljawdah et al., 2022). According to the World Health Organization, malaria is still a dangerous disease that affects 229 million people each year and causes a high rate of mortality (World Health Organization, 2020).

E. camaldulensis belongs to family Myrtaceae and considered a source of biologically active compound s for the treatment of many diseases (Ghalem & Mohamed, 2014; Anigboro et al., 2020). Recently, Anigboro et al. (2020) reported that *E. camaldulensis* could protect against malarial-induced aberrations in liver and renal function in mice infected with *Plasmodium berghei*. In addition, our group reported the antioxidant and immune modulator role of *E. camaldulensis* in mice infected with *P. chabaudi* (Aljawdah et al., 2022)

Although the plant kingdom is a promising source of new active substances, the variability of chemical composition among different species of the same species can affect its quality and bioactivity (Tian et al., 2016). Few studies have addressed this problem or reported differences in composition and/or activity between the same species from different areas. Here, the target is to evaluate the antiplasmodial activity of *E. camaldulensis* harvested from two different habitats (Sandy and muddy). To the best of our knowledge, this is the first report on the comparison of Eucalyptus belonging to the same species but from two distinct geographical areas.

2 Materials and methods

2.1 Preparation of Eucalyptus camaldulensis extracts (ECE)

Leaves were collected from plants growing in muddy and sandy habitats in Qassim, Saudi Arabia. A specialist from King Saud University's herbarium identified the plant. The leaves were air-dried before being ground into powder. The powdered leaves' constituents were then extracted with 70% methanol (Lubbad et al., 2015) . Distilled water was used to dilute ECE from muddy (ECEM) and sandy (ECES) samples for subsequent experiments.

Infrared spectroscopy

According to the method mentioned in Pakkirisamy et al. (2017), we utilized a Nicolet 6700 Fourier-transform infrared spectroscopy (FT-IR) optical spectrometer from ThermoScientific

Received 26 Jan., 2023
Accepted 23 Feb., 2023
[1] *Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt*
[2] *Applied Science Research Center, Applied Science Private University, Amman, Jordan*
[3] *Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia*
[4] *Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia*
[5] *Boehringer-Ingelheim Pharma, Biberach, Germany*
*Corresponding author: mohameddkhil@yahoo.com

(Waltham, MA, United States) for both the ECEM and ECES extracts analyses.

2.2 Determination of phenolic and flavonoid compounds

The total phenolic and flavonoid contents of both ECEM and ECES were determined. Modified Folin-Ciocalteu method using a gallic acid standard curve was carried out to determine total phenolics (Al-Zharani et al., 2019) while Aluminium chloride method using a calibration curve of quercetin was performed to determine total flavonoids (Ghosh et al., 2013).

2.3 Antioxidant activity by DPPH radical scavenging method

Using 2,2-diphenyl-1-picrylhydrazyl (DPPH), the free radical scavenging activity (RSA) of extracts was assessed. In a brief, 80 mL of a methanolic DPPH solution was combined with 20 mL of ECE (1 mg/mL), and the mixture was then incubated for 30 minutes at 25 °C in the dark. At 517 nm, the absorbance was measured, and the RSA was estimated (Ghosh et al., 2013).

2.4 Animals and infection

The normal diet and unlimited water were given to female C57BL/6 mice (9-11 weeks old) during breeding. The experiments were approved by King Saud University's Research Ethics Committee for Laboratory Animal Care (approval no.: KSU-Se-21-77).

2.5 Infection and treatment of mice

P. chabaudi parasites were passage into experimental mice as previously described (Wunderlich et al., 1982). The injected dose (10^6 parasitized erythrocytes) was calculated using a Neubauer chamber, and the *P. chabaudi*-parasitized erythrocytes were injected intraperitoneally into mice (Wunderlich et al., 1982). Giemsa-stained smears of mouse tail blood were prepared to determine *P. chabaudi* induced parasitemia.

According to our previous experiments to determine the suitable dose (see Table 1) and to our recently published study (Aljawdah et al., 2022), we divided mice into the following groups:

Group1 (control non-infected group without treatment), Group 2 (non-infected treated group with 100 mg/kg ECEM), Group 3 (non-infected treated group with 100 mg/kg ECES), Group 4 (infected group without treatment), Group 5 (infected-treated group with 100 mg/kg ECEM),Group 6 (infected treated group with 100 mg/kg ECES), and Group 7 (infected treated group with Chloroquine). The last group was administered 10 mg/kg chloroquine phosphate (CQ) (Sigma-Aldrich, St. Louis, USA) daily for 4 days (Abay et al., 2015).

On day 7 postinfection, mice were sacrificed and blood and spleens were collected. Spleen index was determined as ratio of spleen weight in mg to mouse weight per g.

2.6 Erythrocytes number and hemoglobin content

Blood was collected directly from the heart into heparinized tubes. The number of erythrocytes and haemoglobin content in mouse blood were calculated using the VET-530 CA Medonic blood counter (Stockholm, Sweden).

2.7 Statistical analysis

One-way analysis of variance was used to assess significance, and statistical comparisons between groups were performed using Duncan's test and a statistical package program (SPSS version 17.0). All values are expressed as the mean and standard deviation. All *p*-values were two-tailed, and $p \leq 0.05$ was considered significant for all statistical analyses.

3 Results and discussion

Only a small number of studies have documented variations in species' composition and/or activity across geographical regions. In this study, we assessed the antiplasmodial efficacy of ECE obtained from two distinct, geographically distinct muddy and sandy locations. This is the first study to compare plants extracts from the same genus and species (*Eucalyptus camaldulensis*) but from two different geographical regions.

Tables 1 and 2 were extracted from the IR spectrum table (Merck KGaA, 2023). Infrared spectroscopic evaluation indicated the presence of 13 expected compounds in ECEM and 10

Table 1. FT-IR spectrum of *Eucalyptus camaldulensis* extract from muddy habitat.

Absorption (cm^{-1})	Appearance	Transmittance (%)	Group	Compound Class
3389.80	medium	17.56847	N-H stretching	aliphatic primary amine
2932.18	medium	15.19674	C-H stretching	alkane
1712.21	strong	8.87395	C=O stretching	carboxylic acid
1613.99	strong	8.364901	C=C stretching	α,β-unsaturated ketone
1514.88	strong	7.851239	N-O stretching	nitro compound
1447.62	medium	7.502647	C-H bending	alkane
1352.19	strong	7.008058	S=O stretching	sulfonate
1224.57	strong	6.346636	C-O stretching	vinyl ether
1045.42	strong, broad	5.418147	CO-O-CO stretching	anhydride
871.91	strong	4.518888	C-H bending	1,2,4-trisubstituted
819.57	strong	4.247623	C-H bending	1,4-disubstituted or 1,2,3,4-tetrasubstituted
767.38	Strong	3.977136	C-H bending	Monosubstituted or 1,2- disubstituted
603.07	strong	3.125559	C-Br stretching	halo compound

Table 2. FT-IR spectrum of *Eucalyptus camaldulensis* extract from sandy habitat.

Absorption (cm^{-1})	Appearance	Transmittance (%)	Group	Compound Class
3407.17	strong, broad	20.79017	O-H stretching	alcohol
2931.99	strong, broad	17.89067	N-H stretching	amine salt
1709.87	strong	10.43344	C=O stretching	aliphatic ketone
1614.31	strong	9.850339	C=C stretching	α,β-unsaturated ketone
1515.70	strong	9.248632	N-O stretching	nitro compound
1449.95	medium	8.847433	C-H bending	alkane
1352.00	Strong	8.249753	S=O stretching	Sulfonate or sulfonamide
1043.16	Strong, broad	6.365246	CO-O-CO stretching	anhydride
768.33	Strong	4.688264	C-H bending	Monosubstituted or 1,2- disubstituted
595.89	strong	3.636054	C-I stretching	halo compound

compounds in ECES. Most of these compounds are with strong appearance including groups with N-H, O-H, C-H, C=O, N-H, CO-O-CO, C-I stretching (Tables 1 and 2, Figures S1 and S2 - Supplementary Material). These expected compounds included aliphatic primary amine, α,β-unsaturated ketone, nitro compound, alkane, anhydride, Monosubstituted or 1,2- disubstituted and aliphatic ketones. Horton et al. (2019) reported that phenolic structures play an important role in the bioactivity of compounds with antioxidant activity related to the O-H bond.

P. chabaudi was used as a murine parasite model in this study due to its similarity to the human malaria parasite, *P. falciparum*, to investigate the role of ECE during infection (Mehlhorn, 2014). When comparing the suppression of parasitemia (Figure 1) in after treatment with both ECEM (84.2 ± 3%) and ECES (69.3 ± 2%), we notice the more effectiveness of the extract from muddy habitat. This is due to the higher phenolic and flavonoid content (Table 3) as well as the higher antioxidant activity of ECEM (Figure 2). Several reports owed the reduced parasitemia to treatment with medicinal plants (Wunderlich et al., 2014; Dkhil et al., 2021; Aljawdah et al., 2022). Anigboro et al. (2020) also indicated that *E. camaldulensis* aqueous leaf extracts could protect against *P. berghei* by significantly reducing malarial-induced organ dysfunction due to the presence of active ingredients in the extract. Moreover, flavonoids are able to inhibit the intraerythrocytic growth of the *Plasmodium* where they possess antiplasmodial activity when isolated (Lehane & Saliba, 2008).

The chemical composition of a plant species can be influenced by the location of harvesting, which can affect its quality and bioactivity (Camara et al., 2021). Additionally, Tian et al. (2016) have shown substantial variation in phytochemical components and the bioactivity of same species from various origins.

ECEM and ECES improved the weight loss in mice due to infection with *P. chabaudi* (Figure 3). The decreased weight occurred due to increased parasitemia where animals' loss appetite and suffer from anemia (Dkhil et al., 2021; Aljawdah et al., 2022).

The total number of erythrocytes decreased significantly during infection, but increased after mice were treated with ECEM or ECES (Table 4). Infected mice also had lower haemoglobin levels, but after treatment with ECEM or ECES, they had higher haemoglobin levels, just like the reference drug, chloroquine. (Table 4).

Table 3. Total phenolic and flavonoid compounds of *Eucalyptus camaldulensis* leaf extracts from mud (ECEM) and sand (ECES) habitats.

Extract	Total phenolics (µg/g)	Total flavonoids (µg/g)
ECEM	105.2 ± 3	7.6 ± 0.5
ECES	97 ± 2*	3.2 ± 0.4*

*Significant change between ECEM and ECES at p<0.001.

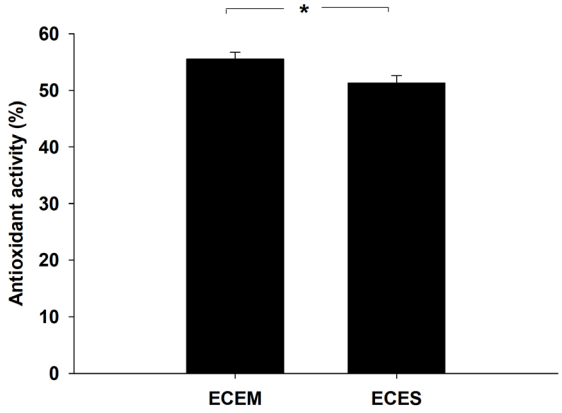

Figure 1. Antioxidant activity of *Eucalyptus camaldulensis* leaf extract from muddy (ECEM) and sandy (ECES) habitat.

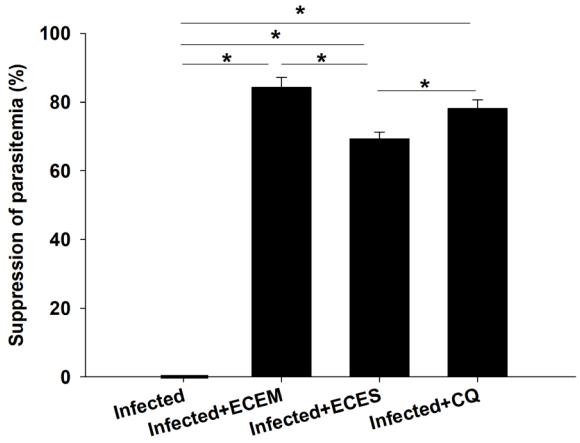

Figure 2. Suppression of parasitemia in mice infected with *P. chabaudi* and treated with *Eucalyptus camaldulensis* leaf extract from muddy (ECEM) and sandy (ECES) habitat. Values are mean ± SD. *Significance against infected group. CQ: Chloroquine.

Table 4. Effect of *Eucalyptus camaldulensis* leaf extract from muddy (ECEM) and sandy (ECES) habitat on the level of erythrocytes and hemoglobin of mice infected with *P. chabaudi*.

Hemoglobin (g/dl)	Erythrocytess (x10^6/mm^3)	Group
14.1 ± 0.4	10.1 ± 0.3	**Control**
14.3 ± 0.6	10.3 ± 0.4	**ECEM**
14.5 ± 0.3	10.1 ± 0.3	**ECES**
9.8 ± 0.8*	6.7 ± 1.6*	**Infected**
12 ± 1*#	7.3 ± 0.5*	**Infected+ECEM**
10.3 ± 1.7*	6.7 ± 1.6*	**Infected+ECES**
13.7 ± 1#	8.6 ± 0.7*	**Infected+CQ**

Values are mean ± SD. *Significance against control group; #Significance against infected group; CQ: Chloroquine.

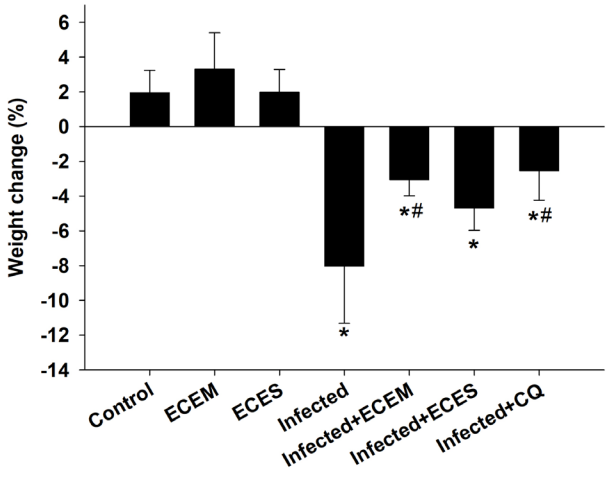

Figure 3. Effect of *Eucalyptus camaldulensis* leaf extract from muddy (ECEM) and sandy (ECES) habitat on weight of mice infected with *P. chabaudi*. Values are mean ± SD. *Significance against control group; #Significance against infected group. CQ: Chloroquine.

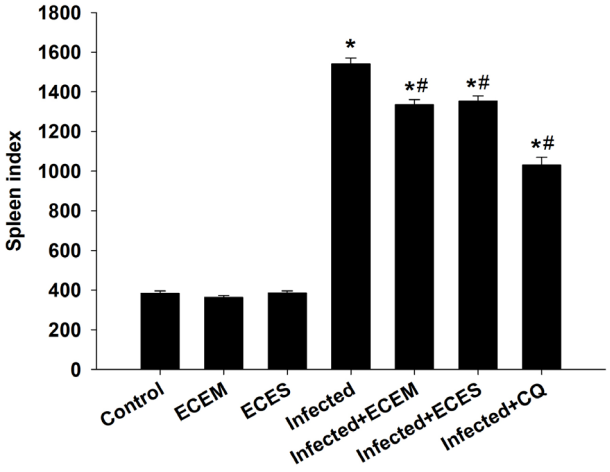

Figure 4. Effect of *Eucalyptus camaldulensis* leaf extract from muddy (ECEM) and sandy (ECES) habitat on spleen index of mice infected with *P. chabaudi*. Values are mean ± SD. *Significance against control group; #Significance against infected group. CQ: Chloroquine.

Hemoglobin is the most direct and sensitive measure for detecting anemia (Quintó et al., 2006). Treatment with ECE could regulate anemia due to the presence of active compounds including phenolics and flavonoids.

The infection induced mice splenomegaly. This was evidenced through the determination of spleen index that reached more than 3-fold increase (Figure 4) when compared to the no-infected group. ECEM and ECES decreased the spleen index nearly to the same extent but in chloroquine treated group the spleen index was reduced to significantly after treatment. The infection induced increase in parasitemia results in increased spleen size (Min-Oo et al., 2004; Dkhil, 2009). During an infection, the spleen plays an important role. With blood-stage malaria, it can clear infected erythrocytes (Del Portillo et al., 2012; White, 2008).

Anemia is thought to be one of the main manifestations of hyperreactive malaria splenomegaly and is most likely the most frequent consequence of malaria (Bryceson et al., 1983). Due to the presence of numerous active phytochemical compounds with therapeutic action, ECE may have improved anaemia and splenomegaly in this study (Lawal et al., 2012).

Metabolite content is heavily influenced by genetic and environmental factors, though the extent of variation caused by these factors varies depending on the type of metabolite. Medicinal plants produce a large number of metabolites with diverse structures, all of which play important roles in plant growth, development, and environmental response (Camara et al., 2021). The study by Zhang et al. (2017) have shown the connection between the effectiveness, chemical components, and distribution of the antimalarial medicinal plant, *Artemisia annua* L. collected from several geographic areas.

The difference in antimalarial and antiplasmodial activity between ECEM and ECES samples could be attributed to harvesting location differences. ECEM was harvested from well-irrigated land, whereas ECES was harvested from sand, which is less irrigated and thus less favorable to optimal plant growth (Traore et al., 2013).

However, since only two habitats were compared in this study, it is evident that *Eucalyptus camaldulensis*, which grows best in muddy habitats, might be employed as an antimalarial agent with the highest efficacy gradients. To determine the molecular mechanisms of the extract's action and to confirm its activity in mouse organs, more research is needed.

Ethical approval

The experiments were approved by the Research Ethics Committee for Laboratory Animal Care at King Saud University (approval no.: KSU-Se-21-77).

Conflict of interest

The authors declare that they have no conflict of interest regarding the content of this article.

Availability of data and material

The data used to support the findings of this study are included within the article.

Author contributions

M.A.D., S.A. and R.A. contributed to study design. M.A.D., F.A.T. and H.M.A.A. contributed to data acquisition. M.A.D., R.A., D.D., F.A.T. and S.A. performed the statistical analysis. All authors revised, improved, read, and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

This study was supported by the Researchers Supporting Project (RSP-2023R3), King Saud University, Riyadh, Saudi Arabia, and also was supported by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2023R96), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

References

Abay, S. M., Lucantoni, L., Dahiya, N., Dori, G., Dembo, E. G., Esposito, F., Lupidi, G., Ogboi, S., Ouédraogo, R. K., Sinisi, A., Taglialatela-Scafati, O., Yerbanga, R. S., Bramucci, M., Quassinti, L., Ouédraogo, J. B., Christophides, G., & Habluetzel, A. (2015). Plasmodium transmission blocking activities of Vernonia amygdalina extracts and isolated compounds. *Malaria Journal*, 14(1), 288. http://dx.doi.org/10.1186/s12936-015-0812-2. PMid:26208861.

Aljawdah, H. M. A., Abdel-Gaber, R., Al-Shaebi, E. M., Thagfan, F. A., Al-Quraishy, S., Qasem, M. A. A., Murshed, M., Mares, M. M., Al-Otaibi, T., Hawsah, M. A., & Dkhil, M. A. (2022). Hepatoprotective activity of *Eucalyptus camaldulensis* extract in murine malaria mediated by suppression of oxidative and inflammatory processes. *Frontiers in Cellular and Infection Microbiology*, 12, 955042. http://dx.doi.org/10.3389/fcimb.2022.955042. PMid:36034714.

Al-Zharani, M., Nasr, F. A., Abutaha, N., Alqahtani, A. S., Noman, O. M., Mubarak, M., & Wadaan, M. A. (2019). Apoptotic induction and anti-migratory effects of Rhazya stricta fruit extracts on a human breast cancer cell line. *Molecules (Basel, Switzerland)*, 24(21), 3968. http://dx.doi.org/10.3390/molecules24213968. PMid:31683960.

Anigboro, A. A., Avwioroko, O. J., & Cholu, C. O. (2020). Phytochemical constituents, antimalarial efficacy, and protective effect of *Eucalyptus camaldulensis* aqueous leaf extract in *Plasmodium berghei*-infected mice. *Preventive Nutrition and Food Science*, 25(1), 58-64. http://dx.doi.org/10.3746/pnf.2020.25.1.58. PMid:32292756.

Bryceson, A., Fakunle, Y. M., Fleming, A. F., Crane, G., Hutt, M. S., de Cock, K. M., Greenwood, B. M., Marsden, P., & Rees, P. (1983). Malaria and splenomegaly. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 77(6), 879. http://dx.doi.org/10.1016/0035-9203(83)90319-X. PMid:6665848.

Camara, A., Haddad, M., Traore, M. S., Chapeland-Leclerc, F., Ruprich-Robert, G., Fourasté, I., Balde, M. A., Royo, J., Parny, M., Batigne, P., Salon, M., Coste, A., Balde, A. M., & Aubouy, A. (2021). Variation in chemical composition and antimalarial activities of two samples of *Terminalia albida* collected from separate sites in Guinea. *BMC Complementary Medicine and Therapies*, 21(1), 64. http://dx.doi.org/10.1186/s12906-021-03231-3. PMid:33588819.

Del Portillo, H. A., Ferrer, M., Brugat, T., Martin-Jaular, L., Langhorne, J., & Lacerda, M. V. (2012). The role of the spleen in malaria. *Cellular Microbiology*, 14(3), 343-355. http://dx.doi.org/10.1111/j.1462-5822.2011.01741.x. PMid:22188297.

Dkhil, M. A. (2009). Apoptotic changes induced in mice splenic tissue due to malaria infection. *Journal of Microbiology, Immunology, and Infection*, 42(1), 13-18. PMid:19424553.

Dkhil, M. A., Al-Quraishy, S., Al-Shaebi, E. M., Abdel-Gaber, R., Thagfan, F. A., & Qasem, M. A. (2021). Medicinal plants as a fight against murine blood-stage malaria. *Saudi Journal of Biological Sciences*, 28(3), 1723-1738. http://dx.doi.org/10.1016/j.sjbs.2020.12.014. PMid:33732056.

Ghalem, B. R., & Mohamed, B. (2014). Antibacterial activity of essential oil of north west Algerian *Eucalyptus camaldulensis* against *Escherichia coli* and *Staphylococcus aureus. Journal of Coastal Life Medicine*, 2(10), 799-804.

Ghosh, S., Derle, A., Ahire, M., More, P., Jagtap, S., Phadatare, S. D., Patil, A. B., Jabgunde, A. M., Sharma, G. K., Shinde, V. S., Pardesi, K., Dhavale, D. D., & Chopade, B. A. (2013). Phytochemical analysis and free radical scavenging activity of medicinal plants *Gnidia glauca* and *Dioscorea bulbifera*. *PLoS One*, 8(12), e82529. http://dx.doi.org/10.1371/journal.pone.0082529. PMid:24367520.

Horton, K. G., Van Doren, B. M., La Sorte, F. A., Cohen, E. B., Clipp, H. L., Buler, J. J., Fink, D., Kelly, J. F., & Farnsworth, A. (2019). Holding steady: Little change in intensity or timing of bird migration over the Gulf of Mexico. *Global Change Biology*, 25(3), 1106-1118. http://dx.doi.org/10.1111/gcb.14540. PMid:30623528.

Lawal, T. O., Adeniyi, B. A., Adegoke, A. O., Franzblau, S. G., & Mahady, G. B. (2012). In vitro susceptibility of Mycobacterium tuberculosis to extracts of *Eucalyptus camaldulensis* and *Eucalyptus torelliana* and isolated compounds. *Pharmaceutical Biology*, 50(1), 92-98. http://dx.doi.org/10.3109/13880209.2011.625953. PMid:22129202.

Lehane, A. M., & Saliba, K. J. (2008). Common dietary flavonoids inhibit the growth of the intraerythrocytic malaria parasite. *BMC Research Notes*, 1(1), 26. http://dx.doi.org/10.1186/1756-0500-1-26. PMid:18710482.

Lubbad, M. Y., Al-Quraishy, S., & Dkhil, M. A. (2015). Antimalarial and antioxidant activities of Indigofera oblongifolia on Plasmodium chabaudi-induced spleen tissue injury in mice. *Parasitology Research*, 114(9), 3431-3438. http://dx.doi.org/10.1007/s00436-015-4568-y. PMid:26109255.

Mehlhorn, H. (2014). *Encyclopedic reference of parasitology* (4th ed.). Heidelberg: Springer.

Merck KGaA. (2023). *IR spectrum table.* Retrieved from sigmaaldrich.com

Min-Oo, G., Fortin, A., Tam, M. F., Gros, P., & Stevenson, M. M. (2004). Phenotypic expression of pyruvate kinase deficiency and protection against malaria in a mouse model. *Genes and Immunity*, 5(3), 168-175. http://dx.doi.org/10.1038/sj.gene.6364069. PMid:15029238.

Newman, D. J., & Cragg, G. M. (2016). Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Products*, 79(3), 629-661. http://dx.doi.org/10.1021/acs.jnatprod.5b01055. PMid:26852623.

Pakkirisamy, M., Kalakandan, S. K., & Ravichandran, K. (2017). Phytochemical screening, GC-MS, FT-IR analysis of methanolic extract of Curcuma caesia Roxb (Black Turmeric). *Pharmacognosy Journal*, 9(6), 952-995. http://dx.doi.org/10.5530/pj.2017.6.149.

Quintó, L., Aponte, J. J., Menéndez, C., Sacarlal, J., Aide, P., Espasa, M., Mandomando, I., Guinovart, C., Macete, E., Hirt, R., Urassa, H., Navia, M. M., Thompson, R., & Alonso, P. L. (2006). Relationship between haemoglobin and haematocrit in the definition of anaemia.

Tropical Medicine & International Health: TM & IH, 11(8), 1295-1302. http://dx.doi.org/10.1111/j.1365-3156.2006.01679.x. PMid:16903892.

Tian, Y. Q., Hu, G. W., & Guo, M. Q. (2016). Components and Anti-HepG2 activity comparison of lycopodium alkaloids from four geographic origins. *Evidence-Based Complementary and Alternative Medicine*, 2016, 4631843. http://dx.doi.org/10.1155/2016/4631843. PMid:27022402.

Traore, M. S., Baldé, M. A., Diallo, M. S., Baldé, E. S., Diané, S., Camara, A., Diallo, A., Balde, A., Keïta, A., Keita, S. M., Oularé, K., Magassouba, F. B., Diakité, I., Diallo, A., Pieters, L., & Baldé, A. M. (2013). Ethnobotanical survey on medicinal plants used by Guinean traditional healers in the treatment of malaria. *Journal of Ethnopharmacology*, 150(3), 1145-1153. http://dx.doi.org/10.1016/j.jep.2013.10.048. PMid:24184265.

White, N. J. (2008). Plasmodium knowlesi: the fifth human malaria parasite. *Clinical Infectious Diseases*, 46(2), 172-173. http://dx.doi.org/10.1086/524889. PMid:18171246.

World Health Organization – WHO. (2020). *World Malaria report*. Geneva: WHO.

Wunderlich, F., Al-Quraishy, S., Steinbrenner, H., Sies, H., & Dkhil, M. A. (2014). Towards identifying novel anti-Eimeria agents: trace elements, vitamins, and plant-based natural products. *Parasitology Research*, 113(10), 3547-3556. http://dx.doi.org/10.1007/s00436-014-4101-8. PMid:25185667.

Wunderlich, F., Stübig, H., & Königk, E. (1982). Development of *Plasmodium chabaudi* in mouse red blood cells: structural properties of the host and parasite membranes. *The Journal of Protozoology*, 29(1), 60-66. http://dx.doi.org/10.1111/j.1550-7408.1982.tb02880.x. PMid:7086713.

Zhang, X., Zhao, Y., Guo, L., Qiu, Z., Huang, L., & Qu, X. (2017). Differences in chemical constituents of *Artemisia annua* L from different geographical regions in China. *PLoS One*, 12(9), e0183047. http://dx.doi.org/10.1371/journal.pone.0183047. PMid:28880869.

Supplementary Material

Supplementary material accompanies this paper.

Figure S1. FT-IR spectrum of Eucalyptus camaldulensis leaf extract from muddy habitat.

Figure S2. FT-IR spectrum of Eucalyptus camaldulensis leaf extract from sandy habitat.

This material is available as part of the online article from https://doi.org/10.1590/fst.006723