Journal of Scientific Research and Studies Vol. 7(2), pp. 30-35, July, 2020
ISSN 2375-8791
Copyright © 2020
Author(s) retain the copyright of this article
http://www.modernrespub.org/jsrs/index.htm

Full Length Research Paper

In vitro activity of extracts from *Dracontium gigas* against *Plasmodium berghei*

Liz Brenes[1]*, Vanessa Bagnarello[2], Misael Chinchilla[2], Idalia Valerio[2], José Bolaños[2] and Mariana Russell[2]

[1]Biology Department of the Research Program for the Sustainable Management of Natural Resources. Universidad de Costa Rica, Alajuela, Costa Rica.
[2]Research Department, Universidad de Ciencias Médicas (UCIMED), San José, Costa Rica.

*Corresponding author. E-mail: ramirezchc@ucimed.com

Accepted 16 June, 2020

In a chemical analysis of several parts of *Dracontium gigas*, we found some components of the terpene family, as well as sterols, flavonoids, saponins and reducing compounds. After a detailed fractionation of leaves and petioles, specific fractions were obtained in the extraction with water, hexane, dichloromethane and ethyl acetate. Those fractions were tested for antimalarial activity using a model with *Plasmodium berghei* (murine malaria), according to the World Health Organization (WHO) recommendation. Petiole dichloromethane and leaf lamina hexane extracts showed IC_{50} activity at 11.64 µg/mL and 14.9 µg/mL, respectively. According to these results, a triplicate analysis of the leaf lamina hexane extract and the petiole dichloromethane extract was performed, and it was found that the petiole dichloromethane fraction reported an activity of 5.56 ± 2 µg/mL, while hexane extract reported an activity of 35.17 ± 3 µg/mL. Given the chemical results obtained, it appears that the activity is due to the chemical components of the terpene and triterpenes family. Future in vivo studies will confirm the importance of *D. gigas* in malaria treatment.

Key words: *Dracontium gigas*, *Plasmodium berghei*, malaria, plant extracts, Costa Rica.

INTRODUCTION

Malaria is caused by several species of *Plasmodium*. The parasite is transmitted to humans through the bites of infected mosquitoes of the genus *Anopheles*. There are many *Plasmodium* species, but only five types cause malaria in humans. These are 1) *Plasmodium falciparum*, which is the most common type of malaria parasite, mainly found in Africa and responsible for most malaria deaths worldwide. 2) *Plasmodium vivax*, regularly found in Asia and Central and South America; this parasite causes milder symptoms than *P. falciparum*, but it stays in the liver for up to 3 years, which can result in relapses. 3) *Plasmodium ovale*, which is fairly uncommon and usually found in West Africa; it can remain inside the liver for several years without producing symptoms, similar to *P. vivax.* 4) *Plasmodium malariae*, which is quite rare and usually only found in Africa. 5) *Plasmodium knowlesi*, which is very rare and found in parts of Southeast Asia; it is associated with non-human primates (Ashley et al., 2018).

After a short time in the liver, the aforementioned parasites grow and multiply in the red blood cells. At regular intervals, usually every 48–72 h, infected blood cells break, releasing more parasites into the blood. Each time a break occurs, the infected person develops a bout of fever, chills and sweating. Even when the bite of *Anopheles* is the principal route of contraction, malaria is also transmitted through blood transfusion and the sharing of needles (Abdullah and Karunamoorthi, 2015), although these methods of contagion are unusual.

Typical symptoms of malaria are moderate to severe shaking chills, high fever, profuse sweating, headache, anemia, muscle pain, convulsions, coma, bloody stools

and urine, and in some cases, death occurs (Ashley et al., 2018).

After diagnosis, the disease must be treated. However, in some cases, the prescribed medication may not clear the infection due to the parasite's resistance to the prescribed drug. If this occurs, more than one medication or a change in medications is necessary. The widespread use of artemisinin-based combination therapies has contributed to substantial declines in the number of malaria-related deaths; however, the emergence of drug resistance threatens to reverse this progress. Of note, *P. falciparum* and *P. vivax* develop resistance more easily than the other parasites (Phillips et al., 2017).

To prevent resistance from becoming an emergency, plant compounds need to be studied, and tropical plants are a good source of new potential antimalarial drugs. The plant *Dracontium gigas*, analyzed in this study, is a possible source of active compounds against *Plasmodium* infection.

The genus *Dracontium* belongs to the Araceae family, the Lasioidae subfamily, and the Alismatales order with 23 species (Zhu and Croat, 2004; Collantes-Díaz et al., 2011; Anonymous, 2013; Rivera-Parada, 2013). Such species are found in several countries, including Costa Rica. *D. gigas*, which is the focus of this study, has been reported by Anonymous (1870), Kusmin (1997), Anonymous (2011), Grayum (2003) and Brenes-Cambronero (2014). The chemical components reported for these plants include flavonoids, terpenes, steroids and coumarins (Velandia and Dayenny, 2009; Collanthes-Diaz et al., 2011; Caro et al., 2017). For *D. gigas*, Zhu (1994) carried out the characterization process in relation to the lectotype and the epitope; however, regarding the chemical components of this plant in Costa Rica, there are no previous studies.

Medically, the components of the *Dracontium* species have been effective in experimental studies on wound healing in mice (Velandia and Dayenny, 2009). *Dracontium* species have also shown an ability to neutralize snake venoms (Otero et al., 2000; Nuñez et al. 2004; Lovera et al., 2006), and in Costa Rica, people in the Bribri and Cabecar communities use this plant to treat *Bothrops asper* bites and other health problems (Kusmin, 1997). However, there is no study of this plant's effectiveness against malaria infection. In fact, although in Costa Rica there are some studies about *Dracontium*, none is related to parasitic infections.

In Costa Rica, *D. gigas* is found in wild form in the Alberto Manuel Brenes Biological Reserve (Spanish acronym: ReBAMB). It has also been successfully cultivated under controlled conditions (Brenes-Cambronero, 2014). Because many studies seeking chemical components that are effective in bacterial infections (Kloucek et al., 2005; Ulloa-Urizar et al., 2015) and in malaria parasites have been performed in plants from ReBAMB (Chinchilla-Carmona et al., 2011; Bagnarello-Madrigal et al., 2018; Alpízar-Cordero et al., 2018), we considered it important to extend these studies to include *D. gigas.* Therefore, we conducted some in vitro analyses using *P. berghei*, an etiological agent of murine malaria, in an experimental model widely recognized by PAHO/WHO organizations; this was the fundamental objective of our present work.

MATERIALS AND METHODS

The samples used in this work came from plants cultured under controlled conditions of temperature, substrate, water supply and careful monitoring of plant growth (Brenes-Cambronero, 2014); they were clones cultured from tubercles of a mother plant found in ReBAMB (10°13′52″N 84°35′43″W) found south of the Cordillera de Tilarán, Costa Rica. In this study, less than 1 kg of petiole and leaf laminas of *D. gigas* were used. These materials were collected on February 9 and August 1, 2018, as well as in October 2019. They were properly packaged, labeled and placed in a portable cooler for transport to the UCIMED. At the laboratory, fresh material was washed to eliminate external contamination and finely cut to facilitate the extraction process.

Phytochemical study

The petiole and leaf lamina crude extract of *D. gigas* was studied by phytochemical screening according to the procedures of Sharapin (2000) with some modifications. Three liquid–liquid ethyl ether extractions were performed with 40 mL of the extract. The etheric extract was concentrated, dried and labeled as (E). The aqueous extract was divided into two parts: AQ1, maintained without any modification. The second aqueous extract was hydrolyzed with 10 mL of HCl (3 mol/L) for 15 m, then a new liquid–liquid process was performed three times with 10 mL ethyl ether and this extract was labeled as AQ2. The corresponding qualitative tests to determine the secondary metabolites were performed for each extract.

Extraction, purification and isolation

Plant crude extracts were macerated for 7 d in 70% ethanol with occasional agitation, and then concentrated on a Büchi R-114 rotary evaporator at 40°C. For the aqueous extract, fractionation with increasing polarity was performed beginning with hexane and followed by dichloromethane and ethyl acetate. All the dried fractions were tested for antiparasitic activity.

Because the dichloromethane fraction showed better activity, it was selected for purification. For this process, a preparative chromatography technique was performed using silica gel 60 W F_{254}S plates, and for the mobile

Table 1. Phytochemical screening of *Dracontium gigas*.

Metabolite	Ethereal extract	Aqueous extract	Hydrolized aqueosus extract
Alcaloids	-	-	-
Flavonoids	-	+	+
Cumarins	-	-	-
Triterpenes and sterols	+		-
Quinones	-	-	-
Tanines	-	-	-
Redutor compounds		+	
Antocianins		-	-
Terpenes	+		-
Saponins		+	

+ Metabolic presence; - Metabolic absence

phase, hexane: ethyl acetate in a 7:3 mixture. The fractions seen at a wavelength of 254 nm were scraped and each fraction was eluted in HPLC grade methanol and filtered on solid phase extraction equipment using Agilent LRC-2OH cartridges. Five fractions were obtained and analyzed in vitro for their antiparasitic activity; the same was done for compound (A) obtained from fraction 4.

Animals

White mice (SWISS-CD1), fed with a commercial concentrate and water *ad libitum*, were used throughout the study and maintained under the international laws endorsed by the Ministry of Science and Technology (Spanish acronym: MICIT).

Parasites

The *P. berghei* strain Vincke and Lips (ATCC 30090) was maintained and passed weekly in at least five mice.

Experimental model for studying antiparasitic activity

A total of 10^5 to 10^6 infected red blood cells were cultured in RPMI medium supplemented with 10% bovine fetal serum (RPMI-BFS) in multi-well plates. For the activity test, 50 µL of plant extracts or fractionated chemical components in different dilutions were added to the corresponding wells, as well as to the positive (0.4% chloroquine) and negative control (only diluent). Red blood cell cultures were kept in an anaerobic box using a paraffin candle to obtain the proper gas concentration to incubate them at 37°C for 24 h. After this incubation period, we prepared smears in slides from the material in each well, which were then stained with Giemsa dye. The percentage of infected erythrocytes and squizonts was determined to compare the inhibition found in the negative controls with that observed in the extracts (Deharo et al., 2000). According to the recommendations of Rasoanaivo et al. (1992), the extracts were classified as very active (IC_{50} 1 to 10 µg/mL), active (IC_{50} more than 10 to 50 µg/mL), suspicious (IC_{50} more than 50 to 100 µg/mL) and negative (above IC_{50} 100 µg/mL).

This study is currently limited to the geographical area indicated above, with an eventual target population of all human beings infected with malaria.

Statistical analysis

The previously positive fractionation test was repeated three times and analyzed using the T test. Any probability less than or equal to 0.05 was considered significant. The analysis was carried out with the help of the statistical program SPSS version 19.

RESULTS

Phytochemical screening of *D. gigas* showed the presence of terpenes and triterpenes in the ethereal extract, and flavonoids and saponines in the aqueous extracts; some other usual compounds were also found in the aqueous extracts (Table 1). When we studied the antiparasitic effect of the fractionation of the crude extracts of the petiole and the leaf lamina of this plant against *P. berghei*, the active inhibition (IC_{50} 11.64 µg/mL and 14.90 µg/mL) of the petiole extract and the leaf lamina was detected, respectively (Table 2). However, in previous studies, unfractionated crude extracts was without significant activity against the parasite.

In the IC_{50} triplicate study of the fractionation of the crude extract, the dichlomethane fraction of the petiole reported an activity of 5.56 ±2 µg/mL, while hexane extract had an activity of 35.17 ±3 µg/mL (Table 3).

Table 2. Antiparasitic activity (IC_{50}) of petiole and leaf crude extrat of *Dracontium gigas.*

Part of the plant	Extract	*Plasmodium berghei* IC_{50} (µg/mL)
Petiole	Hexane	98.77
	Dichloromethane	11.64
	Ethyl Acetate	201.31
	Aqueous	10182
Leaf lamina	Hexane	14.90
	Dichloromethane	100.66
	Ethyl Acetate	152.41
	Aqueous	4021.25

Table 3. Study in triplicate of antiparasitic activity of petiole and leaf lamine of *Dracontium gigas.*

Part of the plant	Extract	*Plasmodium berghei* IC_{50} (µg/mL)
Petiole	Dichloromethane	5.56 ±2
Leaf lamina	Hexane	35.17±3

DISCUSSION

Malaria is an important disease worldwide, with a WHO report (2019) indicating high percentages of infection, especially in tropical countries. In addition, owing to globalization and the ease of transportation between countries on all continents, it is possible to find malaria in humans, even where transmitters are not present. However, although chloroquine has been the drug of choice for this disease, *Plasmodium* species have developed high resistance to it (Bloland, 2001; Anstey et al., 2009). This induces many researchers to seek alternative chemical products obtained from other sources, especially plants (Deharo et al., 2000). The greatest success was obtained with *Artemissia annua*, with chemical components that have been approved by WHO to be used alone or in combination with other drugs in the treatment of malaria (Kakkilaya, 2019; WHO, 2010). Many other studies have been conducted in plants (Kayser et al., 2003; Okello and Kang, 2019; Kojom Foko et al., 2019). In Costa Rica, our group has reported the activity of several plants (Chinchilla-Carmona et al., 2011; Chinchilla et al., 2012), and we are studying some of the active components described experimentally in animals. The present study is another contribution to this field, which was performed in *D. gigas* from ReBAMB, where similar studies have been performed. Because this plant could be cultured under controlled conditions outside the forest, the experimental procedures were more easily conducted.

An interesting observation was that the crude extract did not show a good antiparasitic effect, although the fractions of the same extracts presented very active and active inhibitory effects; this inconsistency has also been observed in other plant studies (Mambe et al., 2019). This inconsistency is probably because other components present in the crude extract block the antiparasitic activity of the active components.

Although we did not find studies of *D. gigas* related to antiparasitic effects, there is some information about certain antibacterial activities found in the ethanol and dichloromethane extracts of this plant (Manrique-Holguín, 2017). These data are in agreement with our results because inhibition of *P. berghei* was found in the dichloromethane fraction, as well as in the hexane fraction, which suggests that terpenes and triterpenes are responsible for this effect.

The count of the number of schizonts in the dilution values at which the IC_{50} was calculated was less than that obtained in the negative control. The control presents an average of 22 ± 2 schizonts, while the petiole averages are 6.33 (lowest dilution) and 13.33 (highest dilution); and for the leaf lamina extract, the averages are 4.32 (lowest dilution) and 22.33 (highest dilution). The T-test for these values shows statistically significant differences between the extracts and the control, being for petiole $p < 0.001$ and for leaf $p < 0.002$.

The IC_{50} of petiole was 5.56 µg/mL, while that of the leaf lamina was 35.17 µg / mL, whose T-test yielded $p < 0.003$, indicating that a smaller amount of petiole extract is needed than the leaf lamina to reach IC_{50}.

Conclusions

These experiments prove that *D. gigas* found in Costa

Rica contains chemical components, probably from the terpenes and triterpenes family, which can inhibit the in vitro multiplication of *P. berghei*. This finding opens up the possibility of performing other studies to demonstrate whether this activity also occurs in vivo, an aspect that we are already examining.

As recommendations, we suggest determining the final chemical characterization of the active components and performing some experiments in animals to determine the in vivo effect of these compounds.

Acknowledgments

This work was supported by the Ministry of Science and Technology (MICIT) and Science and Technology Council (Spanish acronym: CONICIT) through the projects FV-018-2007 and FI-490-11 and under the Research Coordination of the University of Costa Rica's western region. We thank Laura Valerio, the animal care coordinator, and the general assistance of Jimmy Ramirez.

REFERENCES

Abdullah S, Karunamoorthi K (2015). Malaria and blood transfusion: major issues of blood safety in malaria-endemic countries and strategies for mitigating the risk of *Plasmodium* parasites. Parasitol. Res. 115(1):35–47.

Alpízar-Cordero J, Bagnarello-Madrigal V, Rodríguez-Chaves D, Cordero-Villalobos M, Chinchilla-Carmona M, Valerio-Campos I, Sánchez-Porras R (2018). Phytochemical study on *Ticodendrom incognitum* (Ticodendraceae) and determination of its antimalarial activity in vitro. J. Plant Sci. Crop prot. 1(2).

Anonymous (1870). *Dracontium gigas* (Seem.). Engl. Monogr. Phan. 2:284.

Anonymous (2011). *Dracontium gigas* (Seem.) Engl. This species is accepted, and its native range is Nicaragua to Costa Rica. Http://www.kew.org/science-conservation

Anonymous (2013). Consulted on February 11, 2020. http://catearaceae.myspecies.Info/sites/catearaceae.myspecies.info/files/*Dracontium*.pdf

Anstey JR, Russell B, Yeo T, Price RN (2009). The pathophysiology of vivax malaria. Trend. Parasitol. 25:220–227.

Ashley EA, Pyae PA, Woodrow CJ (2018). *Malaria.* The Lancet, 391(10130):1608–1621.

Bagnarello-Madrigal V, Rodríguez-Chaves D, Alpízar-Cordero J, Calvo-Vargas A, Cordero-Villalobos M, Chinchilla-Carmona M, Valerio-Campos I, Sánchez-Porras R (2018). Antimalarial activity of alkaloid mixture isolated from the tender leaves of the *Nectandra membranacea* (Laureaceae). J. Life Sci. 12:134–140.

Bloland PB (2001). Drug resistance in malaria. WHO/CDS/CSR7DRS.

Brenes-Cambronero L (2014). Tuber sprouting, growth and phenology of *Dracontium gigas*, San Ramón, Costa Rica. Rev. Pensamiento Actual. 14(23).

Caro D, Ocampo Y, Castro J, Barrios L, Salas R (2017). Protective effect of *Dracontium dubium* against *Bothrops asper* venom. Biomed. Pharmacother. 89:1105–1114.

Chinchilla M, Valerio I, Sánchez R, Mora V, Bagnarello V, Martínez L, González A, Vanegas JC, Apéstegui A (2012). In vitro antimalarical activity of extracts of some plants from a biological reserve in Costa Rica. Rev. Biol. Trop. 60(2):881–891.

Chinchilla-Carmona M, Valerio-Campos I, Sánchez-Porras R, Mora-Chaves V, Bagnarello-Madrigal V, Martínez-Esquivel L, González-Paniagua A, Vanegas-Pisa JC (2011). In vivo evaluation of antimalarial activity of 25 plants from a Biological Conservation Reserve of Costa Rica. Rev. Chilena de Historia Natural 84:115–123.

Collantes-Diaz IE, Goncalves EG, Yoshida M (2011). Chemical components of tuber of *Dracontium spruceanum* (Schott) G. Zhu ex *Dracontium loretense* Krause (Araceae). Rev. Soc. Quim. Perú. 77(2):117–126.

Deharo E, Gautret PH, Muñoz V, Savain M. (2000) Laboratory techniques for selection of antimalarial substances. Imprenta Pérez, La Paz, Bolivia.

Grayum MH (2003). Araceae. Pp 59. Manual of Plants of Costa Rica, Volume II, Gymnosperms and Monocotyledons, D.E. Zamora (eds.) Missouri Botanical Garden Press, U.S.A.

Kakkilaya BS (2019). Malaria site. URL https://www.malariasite.com/history-control/ (consulted February, 2020).

Kayser O, Kiderien AF, Croft SL (2003). Natural products as antiparasitic drugs, Parasitol. Res. 90:S55–S62.

Kloucek P, Polesny Z, Svobodova B, Vlkova E, Kokoska L (2005). Antibacterial screening of some Peruvian medicinal plants used in Callería District. J. Ethnopharmacol. 99(2):309–12.

Kojom Foko LP, Meva EF, Eboumbou Moukoko CE, Ntoumba AA, Ngaha Njila MI, Ebanda Kedi PB, Ayong L, Lehman LG (2019). A systematic review on anti-malarial drug discovery and antiplasmodial potential of green synthesis mediated metal nanoparticles: overview, challenges and future perspectives. Malar. J. 18(1):337. https://doi.org/10.1186/s12936-019-2974-9

Kusmin VN (1997). *Dracontium sp*. (Araceae): An ethnobotanical study with the cabécar indigenous of Costa Rica. Adv. Rafael Ocampo. ACM Trop Field Res Costa Rica.

Lovera A, Bonilla C, Hidalgo J. (2006). Neutralizing effect of the aqueous extract of *Dracontium loretense* (jergón sacha) on the lethal activity of the *Bothrops atrox* venom. Rev. Per. Med. Exp. Salud Publica. 23(3):177–181.

Mambe FT, Na-Iya J, Fotso GW, Ashu F, Ngameni B, Ngadjui BT, Beng VP, Kuete V (2019). Antibacterial and antibiotic modifying potential of crude extracts, fractions, and compounds from *Acacia polyacantha* Willd. against MDR gram-negative bacteria. Evid. Based Complement. Alternat. Med. Article ID 7507549. 13 pages https://doi.org/10.1155/2019/7507549

Manrique-Holguín PC (0000-0001-8210-6922) (2017). In vitro evaluation of the antimicrobial and cytotoxic effects of methanolic extract of *Dracontium loretense* (jergón sacha) on *Candida albicans* (ATCC®10231TM), *Streptococcus mutans* (ATCC®25175TM) and *Streptococcus sanguinis* (ATCC®10556TM)strains. Universidad Peruana de Ciencias Aplicadas (UPC). Retrieved from http://hdl.handle.net/10757/622864

Núñez V, Otero R, Barona J, Saldarriaga M, Osorio RG, Fonnegra R, Jiménez SL, Díaz A, Quintana JC (2004). Neutralization of the edema-forming, defibrinating and coagulant effects of *Bothrops asper* venom by extracts of plants used by healers in Colombia. Braz. J. Med. Biol. Res. 37(7):969–977.

Okello D, Kang Y (2019). Exploring antimalarial herbal plants across communities in Uganda based on electronic data. Evid. Based Complement. Alternat. Med. Article ID 3057180, 27 pages https://doi.org/10.1155/2019/3057180.

Otero R, Núñez V, Jiménez SL, Fonnegra R, Osorio RG, García ME, Díaz A (2000). Snakebites and ethnobotany in the northwest region of Colombia: Part II: neutralization of lethal and enzymatic effects of *Bothrops atrox* venom. J. Ethnopharmacol. 71(3):505–51.

Phillips MA, Burrows JN, Manyando C, van Huijsduijnen RH, Van Voorhis WC, Wells TNC (2017). Malaria. Nat. Rev. Dis. Primers 3:17050.

Rasoanaivo P, Petitjean A, Ratsimamangaurverg S, Rakoto-Ratsimamanga A (1992). Medicinal plants used to treat malaria in Madagascar. J. Ethnopharmacol. 37:117–127.

Rivera-Parada LL (2013). Phytochemical, pharmaceutical and food characterization of "culebrera indian potato" (*Dracontium spruceanum* (Schott) G.h. Zhu, Araceae) y Sande (*Brosimum utile* (Kunth) Oken, Moraceae) from Botanical Garden of Medicinal plants of CEA de CORPOAMAZONIA, Mocoa, Putumayo.

Sharapin N (2000). Fundamentals of phytotherapeutic product technology. CYTED, Convenio Andrés Bello. Bogotá, Colombia. 247.

Ulloa-Urizar G, Aguilar-Luis MA, De Lama-Odria María del Carmen, Camarena-Lizarzaburu J, Del Valle-Mendoza J (2015). Antibacterial activity of five Peruvian medicinal plants *Pseudomonas aeruginosa*. Asian Pac. J. Trop. Biomed. 5(11):928–931.

Velandia P, Dayenny A (2009). Evaluation of the wound healing activity and phytochemistry characterization of *Dracontium croatii*. Tesis de Maestría Departamento de Farmacia, Universidad Nacional de Colombia. 77.

WHO Library Cataloguing-in-Publication Data Good procurement practices for artemisinin-based antimalarial medicines. 1.Antimalarials – supply and distribution. 2.Artemisinins – supply and distribution. 3.Antimalarials – standards. 4.Quality control. I.World Health Organization. ISBN 978 92 4 159892 7 (NLM classification: QV 256)

Zhu G (1994). Lectotypification and epitypification of *Dracontium gigas* (Seemann) Engler (*Araceae*). Novon 4:404–407.

Zhu G, Croat TB (2004). A revision of the genus *Dracontium* (Araceae). Ann. Missouri Bot. Gard. 91:59–667.