In vitro antiplasmodial activity of twelve plants from the Colombian North coast with low cytotoxicity

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Research

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Abstract

Background

Plants are an important option in the treatment of malaria, especially in endemic regions, and are a less expensive and more accessible alternative with a lower risk of toxicity. Colombia has a great diversity of plants, and the evaluation of natural extracts could result in the discovery of new compounds for the development of antimalarial drugs. The objective of this work was to evaluate the in vitro antiplasmodial activity and the cytotoxicity of plant extracts from the Colombian North coast against *P. falciparum*.

Methods

The antiplasmodial activity of twelve selected plant species from the Colombian North coast used in traditional medicine were evaluated through in vitro cultures of *P. falciparum*, and their cytotoxicity in human cells was determined. Plant extracts with high antiplasmodial activity were subjected to preliminary phytochemical screening.

Results

Five extracts were found to have promising antiplasmodial activity: *Bursera simaruba* (Burseraceae) (bark), *Guazuma ulmifolia* Lam. (Malvaceae) (whole plant), *Murraya exotica* L. (Rutaceae) (leaves), *Hippomane mancinella* L. (Euphorbiaceae) (seeds) and *Capparis odoratissima* Jacq. (Capparaceae) (leaves), presenting IC$_{50}$ values between 1 and 9 µg/ml. These active plant extracts did not show cytotoxic effects on mononuclear cells or haemolytic activity in healthy human erythrocytes compared to the no extract control.

Conclusions

The results obtained from this in vitro preliminary study of the antiplasmodial activity suggest that the active plant extracts from the Colombian North coast are promising for future bioassay-guided fractionation to allow the isolation of active compounds and to understand their mechanism of action against *Plasmodium spp*.

Background

Malaria, a parasitic infection caused by the genus *Plasmodium* that is transmitted through the bite of the *Anopheles* mosquito vector, remains a life-threatening disease globally. According to the WHO, there were approximately 228 million cases of malaria worldwide in 2018 with an estimated 405,000 deaths; additionally, malaria especially affects children under the age of five [1]. In Latin America, it is estimated that 36.5% of the total population lives in ecological conditions favourable to malaria transmission. Colombia, Brazil, Venezuela and Peru account for the highest number of malaria cases per year in Latin America [2]. In Colombia, malaria shows cyclic epidemic behavior [3], and 78513 total cases of malaria were reported in 2019, of which 50.3% corresponded to *Plasmodium falciparum* and 48.6% corresponded to *Plasmodium vivax*, demonstrating the critical presence of these two species in this country [4].
With the resurgence of resistances to antimalarial as a serious problem of biological origin, the advances made so far in preventing and controlling this disease may be threatened in the near future. The accelerated resistance of the parasite towards antimalarial drugs puts the future efficacy of artemisinin-based combination therapies (ACTs) at risk, which is the current scheme and the first line of treatment proposed by the WHO for treatment against malaria complicated by *P. falciparum* [5].

Approximately 80% of the world’s population depends on drugs extracted from plants for their basic health needs. The preparations and infusions derived from natural extracts are fundamental in traditional medicine, mainly in rural areas, where it is often the treatment of first choice with greater access [6].

Compounds derived from plants have been the central point for the development of antimalarial treatments, and two such compounds emerged from South America. Quinine, an active ingredient from the bark of the Cinchona tree, became the first compound with antimalarial activity, from which chloroquine, one of the most widely used antimalarial until recently, was later designed [7].

Likewise, lapachol, belonging to the chemical class of naphthoquinones and used in the nineteenth century for the treatment of fever in South America, was isolated for the first time from *Tabebuia avellanedae* Lorentz ex Griseb. (Bignoniaceae). Later, after chemical optimization, atovaquone was obtained and is currently used in association with proguanil for malaria prophylaxis and treatment [8, 9].

Ethnobotanical studies carried out in various countries have helped to identify a variety of plants that are used in traditional medicine against the symptoms associated with malaria. The Amazon region has great botanical diversity, and research on the antimalarial activity of plant species in this region in countries such as Brazil, Colombia, and Bolivia has demonstrated the potential of local traditional medicinal practices as sources of powerful extracts [10, 11]. Traditional remedies are an integral part of Colombian culture. Various studies of the ethnopharmacology and use of popular medicine among the population of the Atlantic Coast of Colombia, specifically in the Department of Bolívar, have shown many perspectives in the search for new drugs based on local uses of medicinal plants [12].

To date, there is no registered and effective vaccine that can be applied to the population as a preventive measure to control or eradicate malaria. Therefore, chemotherapy continues to be the main measure to combat the disease, and due to increasing drug resistance, the discovery of new therapeutic options is necessary [13].

In this work, the *in vitro* antiplasmodial activity of twelve plants from the Colombian North coast used in traditional medicine was evaluated. According to the results, extracts from five plant species displayed suitable and interesting activity against the *P. falciparum*: *Bursera simaruba* (Burseraceae) (bark), *Guazuma ulmifolia* Lam. (Malvaceae) (whole plant), *Murraya exotica* L. (Rutaceae) (leaves), *Hippomane mancinella* L. (Euphorbiaceae) (seeds) and *Capparis odoratissima* Jacq. (Capparaceae) (leaves), presenting IC₅₀ values < 10 µg/mL. The results obtained from this preliminary study suggest that the extracts tested are promising for future bio-directed assays that could allow the isolation of active compounds to further unravel their mechanism of action against *Plasmodium*.

**Methods**

**Selection and collection of plant material**
Plant species from the north coast of Colombia used in this region as traditional medicine were collected for this study. Twelve species were selected from a bibliographic database review in which they had no or few previous reports of antimalarial activity (Table 1). Voucher specimens were collected and numbered during the interviews. Specimen identification was carried out by comparison with authentic samples. The vouchers were deposited in the Botanical Garden in Cartagena (JBC) and the Herbarium of the National University of Colombia (COL).

Table 1 Plant species from the Colombian North Coast selected for the in vitro study of antiplasmodial activity

| Scientific name                          | Common local name | Family                | Voucher number |
|-------------------------------------------|-------------------|-----------------------|----------------|
| *Bursera graveolens* Kunth. (Burseraceae) | Caraña            | Burseraceae           | [JBC 5115]     |
| *Bursera simaruba* L. (Burseraceae)      | Almácigo          | Burseraceae           | [JBC 5115]     |
| *Cardiospermum grandiflorum* Sw. (Sapindaceae) | Topo-topo        | Sapindaceae           | [JBC 1452]     |
| *Capparis odoratissima* Jacq. (Capparaceae) | Olivo             | Capparidaceae         | [JBC 1492]     |
| *Chenopodium ambrosioides* L. (Amaranthaceae) | Paico, Hierba santa | Chenopodiaceae     | [JBC 4005]     |
| *Coccoloba uvifera* (L.) L. (Polygonaceae) | Uvita de playa    | Polygonaceae          | [JBC 4593]     |
| *Guaiaicum officinale* L. (Zygophyllaceae) | Guayacán          | Zygophyllaceae        | [JBC 2507]     |
| *Guazuma ulmifolia* Lam. (Malvaceae)      | Guácimo           | Sterculiaceae         | [JBC 4539]     |
| *Gustavia superba* (Kunth) O.Berg (Lecythidaceae) | Membrillo        | Lecythidaceae        | [JBC 1382]     |
| *Hippomane mancinella* L. (Euphorbiaceae) | Manzanillo        | Euphorbiaceae        | [JBC 2478]     |
| *Hyptis Capitata* Jacq. (Lamiaceae)       | Botón negro       | Lamiaceae             | [JBC 1389]     |
| *Murraya exotica* L. (Rutaceae)           | Azahar de la India | Rutaceae              | [COL 538418]   |

Preparation of the plant extracts

Vegetable samples were dried for two days at a temperature of 40 °C in an oven with air circulation. After this period, the plant material was ground by mechanical methods using a blade mill. Extracts of the different organs of the plants were obtained from the continuous maceration of 100 g of pulverized material in 96% ethanol in a clean, dry glass bottle, which was kept at room temperature. The extraction was repeated until the plant material was exhausted; after this time, the extracts were filtered and concentrated under reduced pressure in a rotary evaporator [14]. Finally, after weighing, the obtained extracts were dissolved with the smallest possible volume of dimethyl sulfoxide (DMSO) and then diluted as required in culture medium (RPMI 1640) to prepare the different concentrations of the extracts for the biological assays. The final DMSO concentration was never greater than 0.05% to avoid carry-over (solvent) effects [15].

In vitro culture of *Plasmodium falciparum*
Chloroquine-resistant *P. falciparum* strain Dd2 (clone MRA-150) was used for this study. A detailed description of the culture and synchronization methods used has been reported previously [16].

**In vitro antiplasmodial activity of the plant extracts**

A microfluorimetric DNA-based assay was used to monitor parasite growth inhibition with different concentrations of the plant extracts [17]. Synchronized rings from stock cultures were used to test serial dilutions of plant extracts ranging from 100 µg/mL to 0.01 µg/mL in 96-well culture microplates to obtain 2% haematocrit and 1% parasitaemia. Subsequently, the parasite was allowed to grow for 48 hours in 5% CO₂ at 37 °C. After incubation, the microplates were centrifuged at 600 × g for 10 minutes and re-suspended in saponin (0.15%, w/v in phosphate-buffered saline) to lyse the erythrocytes and release the malaria parasites. To eliminate all traces of haemoglobin, the pellet was washed by the addition of 200 µL of PBS followed by centrifugation at 600 × g. The washing step was repeated twice to ensure the complete removal of haemoglobin. Finally, the pellets were re-suspended in 100 µL of PBS and mixed with 100 µL of PicoGreen. The plates were incubated for 30-60 minutes in the dark, and the fluorescence intensity was measured at 485 nm excitation and 528 nm emission. Each test also included an untreated control, and chloroquine was used as the reference drug. Growth inhibition was calculated as previously described [17]. Three independent experiments were performed in triplicate to determine the antiplasmodial activity of each extract. The 50% inhibitory concentration (IC₅₀) of plant extracts against *Plasmodium* growth was determined using Sigma Plot software.

**In vitro cytotoxicity test of the plant extracts on human peripheral blood mononuclear cells**

The cytotoxic activity of plant extracts was evaluated by the trypan blue dye exclusion method on human peripheral blood mononuclear cells (PBMCs) [18]. The dye exclusion test was used to determine the number of viable cells present in a cell suspension. These cells were obtained from blood of healthy donors using Ficoll-Hypaque density gradients. A total of 6×10⁵ cells per well were mixed in triplicate for each tested concentration of the plant extracts (range 1000 µg/mL to 0.01 µg/mL) and controls in RPMI 1640 supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin for a total volume of 200 µL in 96-well microplates. Cells were immediately incubated for 48 hours at 37 °C in a humid atmosphere with 5% CO₂ [19]. At the end of incubation, 100 µL of trypan blue dye was added to 100 µL of cell suspension. Then, viable cells were counted for each replicate at each extract concentration and for the controls. Three independent experiments were performed in triplicate to determine the cytotoxicity of each extract. The 50% lethal concentration (LC₅₀) values of the plant extracts in PBMCs was determined using Sigma Plot software.

**Haemolysis assay of the plant extracts**

The potential haemolytic effect of those plant extracts with promising antiplasmodial activity was investigated by incubating them with healthy human erythrocytes in 96-well microplates to determine absorbance at 540nm that corresponds to the released haemoglobin in supernatants.

For these tests, erythrocytes at 2% haematocrit were incubated with different concentrations of the plant extracts in a concentration range of 1-100 µg/mL. A 2% haematocrit control solution was prepared in distilled water (100% relative haemolysis) as previously described [20]. The results are expressed as a percentage of relative haemolysis compared to the 100% haemolysis control.
**Morphology of the *P. falciparum* cultures after treatment with the plant extracts**

The morphology of the parasite from each culture at different concentrations of the plant extracts, as described in the previous section, was evaluated by microscopic analysis of thin blood smears stained with Wright's stain after incubation. Stage-specific development was assessed by examining a minimum of 1000 parasitized cells on each smear for the differential counting of rings, trophozoites, schizonts, and pyknotic forms whose developmental stage could not be established. The fraction in each group was calculated as a percentage of the total parasitized cells. Parasitaemia was measured by counting 1000 red cells and is reported as the percent of parasitized erythrocytes [21]. Smears from plant extract-free cultures were used as a control.

**Phytochemical screening**

Plant extracts with favourable antiplasmodial activity were subjected to a preliminary phytochemical screen by carrying out identification tests for each specific group of secondary metabolites, such as alkaloids (Dragendorff’s reagent, Wagner's reagent, flavonoids (FeCl3 reagent in 1% EtOH), tannins (FeCl3 assay), coumarins (5% KOH reagent in EtOH, Shinoda assay), saponins (vanillin-sulfuric acid reagent), triterpenes and sterols (Liebermann-Burchard and Salkowski test), quinones (Bornträger test) and cardiac glycosides (Benedict's reagent), as previously described in other studies [14].

**Results**

**In vitro antiplasmodial and cytotoxic activity of the plant extracts studied**

The *in vitro* antiplasmodial activity of twelve plant extracts from the north coast of Colombia was evaluated in the Dd2 strain of *P. falciparum*. Results are described in detail in Table 2. Chloroquine was used as a control, with an IC₅₀ of 0.05 µg/mL against strain Dd2 (chloroquine-resistant).

**Table 2** Antiplasmodial activity and cytotoxicity values of selected plant extracts from the north coast of Colombia
| Scientific name   | Plant part used | IC<sub>50</sub><sup>b</sup> against the Dd2 strain [µg/mL] | Antiplasmodial activity | Cytotoxicity LC<sub>50</sub><sup>c</sup> [µg/mL] | Selectivity index SI |
|-------------------|-----------------|-------------------------------------------------|--------------------------|---------------------------------|---------------------|
| *B. simaruba*     | bark            | 1.2 ± 0.16                                      | high                     | 369.4 ± 17.2                    | 307.8               |
| *G. ulmifolia*    | whole plant     | 3 ± 1.40                                        | high                     | 272.9 ± 15.4                    | 91.0                |
| *M. exótica*      | leaves          | 3.1 ± 0.21                                      | high                     | 351.6 ± 3.5                     | 113.4               |
| *H. mancinella*   | seed            | 4.4 ± 1.30                                      | high                     | 186.8 ± 1.7                     | 42.5                |
| *C. odoratissima* | leaves          | 8.8 ± 0.84                                      | good                     | 298.8 ± 13.3                    | 34.0                |
| *H. Capitata*     | leaves          | 14.1 ± 2.13                                     | moderate                 | 312.4 ± 12.6                    | 22.2                |
| *G. officinale*   | leaves          | 15.7 ± 3.20                                     | moderate                 | 301.6 ± 5.3                     | 19.2                |
| *G. superba*      | leaves          | 19.9 ± 0.40                                     | moderate                 | 254.3 ± 4.2                     | 12.8                |
| *B. graveolens*   | bark            | 26.9 ± 0.56                                     | moderate                 | 298.3 ± 8.7                     | 11.1                |
| *C. uvifera*      | whole plant     | 29.6 ± 4.70                                     | moderate                 | 278.6 ± 11.8                    | 9.4                 |
| *C. grandiflorum* | leaves          | 181.8 ± 7.53                                    | inactive                 | 321.5 ± 2.2                     | 1.8                 |
| *C. ambrosioides* | leaves          | 198.7 ± 6.50                                    | inactive                 | 290.1 ± 15.5                    | 1.5                 |

<sup>a</sup> Crude ethanol extracts were used for each part of the plant.

<sup>b</sup> IC<sub>50</sub>: 50% inhibitory concentration against *P. falciparum* strain Dd2. The IC<sub>50</sub> values are expressed as the mean ± standard deviation of three different determinations per experiment.

<sup>c</sup> LC<sub>50</sub>: 50% lethal concentration in human peripheral blood mononuclear cells. The LC<sub>50</sub> values are expressed as the mean ± standard deviation of three different determinations per experiment.

<sup>d</sup> Selectivity index = LC<sub>50</sub>/IC<sub>50</sub>.

According to WHO guidelines, anti-plasmodial activity is classified as follows: highly active with IC<sub>50</sub> < 5 µg/mL, good activity at 5–10 µg/mL, moderate activity at 11–50 µg/mL and inactive at >50 µg/mL [22, 23].
The selectivity index (SI) of each extract is also presented in Table 2. The SI is defined as the ratio of the LC$_{50}$ value in PBMCs to the IC$_{50}$ value against *P. falciparum* [15].

According to these results, we can highlight five extracts with high to good antiplasmodial activity (Table 2): that correspond to *B. simaruba* (bark), *G. ulmifolia* (whole plant), *M. exotica* (leaves), *H. mancinella* (seeds) and *C. odoratissima* (leaves).

High survival rates were found when evaluating each of the extracts in human PBMCs in the concentration range of 100 to 0.01 μg/mL. The LC$_{50}$ values were above 100 μg/mL and much higher than the IC$_{50}$ against *P. falciparum*, showing a low cytotoxicity when compared with the control without treatment. The selectivity index (SI) of the plant extracts was assessed and demonstrated specific antiplasmodial activity rather than toxicity to PBMCs since most of the indices were ≥ 2.

**Haemolytic effects of the active plant extracts**

To evaluate the effects of the active extracts on the structural integrity of erythrocytes, the haemoglobin concentration was determined in a sample of red blood cells exposed to each extract with high antiplasmodial activity (*B. simaruba, G. ulmifolia, M. exotica, H. mancinella* and *C. odoratissima*) after incubation for 48 hours at 37 °C. From the results obtained in this study, none of the extracts tested showed haemolytic activity in healthy erythrocytes in comparison with the control without extract treatment, as seen in Table 3, which suggests low toxicity of the extracts on erythrocytes.

**Table 3** Percentage of haemolysis in erythrocytes treated with the active extracts from the Colombian North coast$^a$
| Plant extracts          | Concentration (µg/mL) | % Haemolysis |
|------------------------|-----------------------|--------------|
| *Bursera simaruba*     | 1                     | 5.5±0.25     |
|                        | 10                    | 5.0±0.22     |
|                        | 100                   | 5.9±1.93     |
| *Guazuma ulmifolia*    | 1                     | 5.5±0.19     |
|                        | 10                    | 5.7±0.38     |
|                        | 100                   | 5.7±0.25     |
| *Murraya exótica*      | 1                     | 5.7±0.29     |
|                        | 10                    | 5.0±0.27     |
|                        | 100                   | 5.7±0.42     |
| *Hippomane mancinella* | 1                     | 5.2±0.83     |
|                        | 10                    | 5.7±1.51     |
|                        | 100                   | 5.5±0.55     |
| *Capparis odoratissima*| 1                     | 5.8±0.34     |
|                        | 10                    | 5.8±0.34     |
|                        | 100                   | 5.7±0.23     |
| Control without treatment | -                      | 5.7±0.23     |
| Positive control       | -                     | 100          |

Erythrocytes were incubated with each extract at concentrations of 1, 10 and 100 µg/mL at 37 °C for 48 hours. Each data point represents the mean ± standard deviation of two independent experiments performed in triplicate against a positive control (100% haemolysis) and a control without treatment. The percentage of haemolysis generated by the extracts compared with the control without treatment did not show statistically significant differences (p> 0.05).

**Phenotype effects of the active plant extracts on the intraerythrocytic stages of *P. falciparum***

Evaluation of the effects on the morphology of the intraerythrocytic stages of the parasite was carried out by microscopic visualization of smears from the sediment of the microcultures exposed to the active plant extracts.
at concentrations of 100, 10 and 1 μg/mL. In this analysis, we found variations in the proportion of *P. falciparum* forms compared to the untreated control (Fig. 1). The presence of pyknotic forms was frequently observed, consisting of the retraction of the nucleus with condensation of the parasite's chromatin in the form of a solid mass, a morphological sign of cell death, which was observed in all three concentrations of the five active extracts studied (Fig. 2).

Likewise, other modifications to the structure of the parasitic stages that refer to alterations in the normal shape of the rings were observed, such as dysmorphism, delayed maturation of trophozoites and the presence of forms with an increased size of the parasitophorous vacuole (Fig. 1 and Fig. 2). These morphological alterations were seen more frequently at the concentrations of 10 and 1 μg/mL (Fig. 1). Accordingly, we can suggest an inhibitory effect of the active extracts on the maturation of the parasite.

On the other hand, we observed that there was a decrease in the percentage of rings at higher concentrations of the extracts of the active plants, thus suggesting inhibitory action from these extracts on the invasive cycle of the parasite; moreover, in all extracts, a low percentage of schizont was observed at the three concentrations tested (Fig. 1). All these morphological alterations point out to the fact that these active extracts, when tested at a concentration close to their IC₅₀ value, cause important phenotypic changes over the parasite cycle.

**Phytochemical screening**

The different chemical identification tests applied to the extracts of the selected species that displayed antiplasmodial activity show the presence of several families of secondary metabolites. Table 4 shows the results of the phytochemical screen.

**Table 4** Phytochemical screen of the active plant species from Colombian North coast against cultures of *P. falciparum* strain Dd2

| Secondary metabolites | Alk | Cum | Tan | CG | Flav | Sap | Tri/Est | Qui |
|-----------------------|-----|-----|-----|----|------|-----|---------|-----|
| **B. simaruba**       | +   | -   | -   | -  | +    | +   | +       | +   |
| **G. ulmifolia**      | -   | +   | +   | -  | +    | -   | +       | -   |
| **M. exótica**        | +   | +   | -   | -  | +    | +   | +       | -   |
| **H. mancinella**     | +   | +   | +   | +  | +    | -   | -       | -   |
| **C. odoratissima**   | +   | +   | -   | -  | -    | -   | +       | -   |

+: Present, -: Not Detected

Alk: alkaloids, Cum: coumarins, Tan: tannins, CG: cardiotonic glycosides, Flav: flavonoids, Sap: saponins, Tri/Est: triterpenes/steroids, and Qui: quinones.

**Discussion**
The accelerated resistance of the parasite against available antimalarial drugs has become one of the greatest difficulties for the control and eradication of malaria. The development of new therapeutic alternatives that meet the requirements of rapid efficacy, minimal toxicity and low cost is essential and in great need worldwide to counteract this disease [24]. Primary screenings for the evaluation of the antimalarial activity of plant species can generate basic information that allows the selection of potential extracts and a deepening of their composition of secondary metabolites and biological activity against the parasite.

In this study, five plant extracts were found with promising activity to inhibit the development of *P. falciparum in vitro*. The selected species are representative of the Colombian North coast and are used in traditional medicine in the region; these species have been part of a chemical study to identify secondary metabolites and, to date, there are few reports on their actions as antimalarial plants (Table 5).

The five species that were active for antiplasmodial activity were: *B. simaruba* (bark), *G. ulmifolia* (whole plant), *M. exotica* (leaves), *H. mancinella* (seeds) and *C. odoratissima* (leaves), which presented IC\textsubscript{50} values < 10 \( \mu \)g/mL. The extract of *B. simaruba* stands out with the lowest IC\textsubscript{50} value of 1.2.

The inhibitory action of the five active extracts with respect to their phenotypic effects on the intraerythrocytic stages of *P. falciparum* generated mostly the presence of the pyknotic form of the parasite at the maximum concentration tested and a small proportion of the ring forms and schizonts during the incubation period, which indicates that the parasite is not capable of completing the intraerythrocytic cycle, probably affecting the invasive stage of new erythrocytes. At concentrations close to the IC\textsubscript{50} values of the active extracts, there were modifications to the structure of the parasitic form, such as delayed maturation of trophozoites and stages with an increased size of the parasitophorous vacuole. These alterations have already been described and are associated with compounds with powerful antimalarial activity [25].
Table 5
Traditional uses and scientific studies that demonstrate the medicinal properties of plant species, tested for antimalarial activity against Chloroquine-resistant *P. falciparum* strain Dd2

| Scientific name | Traditional uses | Biological and Pharmacological Activities |
|-----------------|------------------|------------------------------------------|
| *B. simaruba*   | Gastrointestinal and respiratory diseases, skin rashes, anti-inflammatory, urinary infections [26, 27] | Anti-inflammatory [28], antimicrobial [29], antioxidant [30], anti-herpesvirus [31], antihypertensive [32] |
| *G. ulmifolia*  | Inflammation, coughs, malaria, syphilis, gastritis, dermatitis and gastrointestinal and cardiovascular disorders [12, 26, 33] | Antimicrobial, antiprotozoal, antioxidant, and antidiarreal activities, and cardioprotective effect[33] |
| *M. exótica*    | Pain, Analgesic, Sedative [14, 34] | Antioxidant [35], anticancer [36], anti-inflammatory [37], antiplasmodial and antipyretic [38] |
| *H. mancinella* | Toxic, causes stomatitis, lip lesions, pharyngeal edema, dermatitis and eye lesions [39, 40] | - |
| *C. odoratissima* | Dermatological conditions [14] | - |
| *H. Capitata*   | Dermatological conditions [14] | Anti-cancer [41], anti-HIV [42] |
| *G. officinale* | Anti-syphilitic [43], anti-inflammatory and diaphoretic effects [44] | Anti-inflammatory [45], anti-rheumatic and anti-oxidant [46], anti-cancer [47], antidiabetic and hepatoprotective [48] |
| *G. superba*    | Respiratory diseases [14] | - |
| *B. graveolens* | Wound cleaning, asthma, diarrhea and kidney stones [49], rheumatism [14], high blood pressure, stomach pain [50] | Anti-cancer [51], anti-proliferative [52], spasmolytic and antibacterial [50] |
| *C. uvifera*    | Antidiarrheal, astringent and Asthma [26], Dermatological conditions [14] | Anti-mutagenic, anti-proliferative and antioxidant [53] |
| *C. grandiflorum* | Dermatological conditions [14] | - |
| *C. ambrosioides* | Antiparasitic [14], inflammation, healing, constipation and flu [54] | Antifungal [55], antioxidant and anti-inflammatory [56], vasodilatory [57], antiparasitic and immunomodulatory [58] |

The alterations observed in the stages of the parasite incubated with the active plant extracts suggest that they could share an analogous mechanism of action, possibly due to the common presence of secondary metabolites such as alkaloids and triterpenes/steroids. This hypothesis could be supported by previous research, where ten triterpenoid compounds with antimalarial activity (IC$_{50}$ of 6 to 7 µM) were isolated and tested against *P. berghei* in murine models [59].

Previous studies based on trophozoite maturation delay to continue the invasive cycle of infection suggested that this response could be consequence of inhibition of parasite proteases and phosphatases, according to the activity of maslinic acid and other related triterpenoid molecules, where a parasitostatic effect was suggested [21]. On the other hand, the presence of alkaloids in all of the active extracts tested is also notable. In previous
reports [60], it was shown that guanidine-type alkaloids have a broad bioactivity profile, especially as enzyme inhibitors.

We cannot finally discard that the compounds present in these active plants extracts may act also blocking essential enzymatic pathways for the development of the erythrocyte cycle of the parasite, compromising their survival. Consequently the results of this screening suggest to further pursue fractionation and biological activity tests to isolate the active compounds responsible for this promising inhibitory effect of *P. falciparum* and to disclose their mechanism of action.

**Conclusions**

This study on the *in vitro* antiplasmodial activity and cytotoxicity tests of the plant extracts: *B. simaruba* (bark), *G. ulmifolia* (whole plant), *M. exotica* (leaves), *H. mancinella* (seeds) and *C. odoratissima* (leaves) showed their inhibitory effect on *P. falciparum* growth with nearly negligible cytotoxic effect and no haemolytic damage. Thus, it is suggested that those extracts should contain active molecules responsible for such biological effect on the malaria parasite supporting their future bioassay-guided fractionation to identify the specific compounds to test their activity and to unravel their mechanisms of action against *P. falciparum*, as a potential discovery of new compounds for the development of antimalarial drugs.

**Declarations**

**Ethics approval and consent to participate**

The ethical and scientific procedures of this study were certified by the Ethics Committee of the Faculty of Medicine, University of Cartagena, Colombia (study number 80/5-03-2015).

**Consent for publication**

Not applicable

**Availability of data and materials**

The datasets used 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’ contribution**

CM, FD, JMB, and AD designed the study. SV prepared plant extracts. SV and CM performed the laboratory experiments. SV and CM wrote the first draft. All authors read and approved the final manuscript.
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**Figures**
Figure 1

Percentage of intraerythrocytic P. falciparum stages in the presence of the active plant extracts from the north coast of Colombia. Representation of the percentages of each parasitic stage and the morphological alterations observed (rings, trophozoites, schizonts, pyknotic forms) after treatment with different concentrations of the active extracts evaluated. All parasitaemia percentages decreased as the concentration of the extracts increased. Data were obtained by microscopic analysis of sediment extensions from exposed microcultures stained with Wright's eosin-methylene blue and compared with controls without extract. The fraction of each group was calculated by counting the proportion of cells infected by each specific stage in a total of 103 erythrocytes.
Figure 2

Effects of the active extracts from the Colombian North coast on the development of the parasite. Chloroquine-resistant P. falciparum strain Dd2 cultures incubated for 48 hours compared to the untreated control. Active extracts: (A) B. simaruba (bark), (B) G. ulmifolia (whole plant), (C) M. exotica (leaves), (D) H. mancinella (seeds) and (E) C. odoratissima (leaves) in a concentration range of 1.0-100 µg/mL. The following morphological changes can be observed: pyknotic forms (P), alterations in the normal shape of rings (X), forms with delayed maturation of trophozoites (Y) and an increase in the size of the parasitophorous vacuole (Z).