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Comparative study of potent anti-amoebic and anti-inflammatory activities of different extracts from *Sida rhombifolia* (L)

Sylvain Pechangou Nsangou, Roukayatou Ndiandoukoue Ndam, Emmanuel Mfotie Njoya, Brice E. Enang II, Frederic N. Njayou, Paul Fewou Moundipa

Abstract

Background: *Sida rhombifolia (L)* is a medicinal plant from tropical regions belonging to the *Malvaceae* family and the *plantea* kingdom. It is known for its multiple pharmacological properties. The aim of this work was to evaluate the anti-amoebic, and anti-inflammatory potential of *Sida rhombifolia* aqueous and ethanolic leaf and stem extracts harvested in two localities of Cameroon (Yaoundé and Massangam in the Centre and West regions, respectively). **Methods:** The anti-amoebic activity was evaluated on a polyxenic culture of *Entamoeba histolytica*. A primary macrophage culture activated by *Saccharomyces cereviseae* (SC) was used to evaluate anti-inflammatory potential of the plant extracts. Macrophages were treated with different concentrations (1; 10; 100 and 500 µg/mL) of the extracts for the inhibition of nitric oxide (NO) production and 5-lipoxygenase activity.
Methods: The anti-amoebic activity was evaluated on a polyxenic culture of *Entamoeba histolytica*. A primary macrophage culture activated by *Saccharomyces cereviseae* (SC) was used to evaluate the anti-inflammatory potential of the plant extracts. Macrophages were treated with different concentrations (1; 10; 100 and 500 µg/mL) of the extracts for the inhibition of nitric oxide (NO) production and 5-lipoxygenase activity.
Results: No significant difference was found between the anti-amoebic activity of the ethanolic extract of Yaoundé (Centre region, IC_{50} = 7.71 µg/mL) and that of ethanolic leaf extract from Massagam (West region IC50 = 7.77 µg/mL) after 72 hours. Among the extracts, ethanolic leaf extracts exhibited the highest anti-amoebic activity after 72 hours of treatment. Metronidazole showed better activity (IC_{50} = 5.96 µg/mL) in the same periods of time. Phenolic compounds and flavonoid contents were higher in Yaoundé (Centre region) ethanolic leaf extract, proving that these extracts can prevent certain damages linked to a state of oxidative stress. The ethanolic extracts of the leaves significantly inhibited NO production and 5-lipoxygenase activity in macrophages (IC_{50} of 3.85 µg/mL; 3.99 µg/mL and 36.64 µg/mL; 73.22 µg/mL respectively).
Conclusion: This preliminary study shows that the ethanolic extract of *S. rhombifolia* possesses better anti-amoebic properties and can act at the level of the intestinal mucosa in order to fight against the damage linked to the dysfunction of the inflammatory cells.

Keywords: *Sida rhombifolia*; anti-amoebic; anti-inflammatory.

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Background

Inflammation is a physiological defense mechanism of the body against invasion by pathogens. Its essential role is the elimination of the pathogen and the repair of injured tissue [1]. However, inflammatory reactions can have adverse effects on the body if not regulated. Inflammation is accompanied by the production of various inflammatory mediators such as chemokines, leukotrienes, prostaglandins, and cytokines including Tumor Necrosis Factor (TNF-α), interleukin-1β (IL-1β), interferon-ɣ (INF-ɣ). Pro-inflammatory cytokines are widely implicated in pathologies, amplifying the inflammatory response, cell hyper-proliferation, and tissue degradation [2]. Depending on the organ, several types of inflammation can be distinguished. Intestinal inflammation is a defensive response of the immune system at the level of the intestinal wall due to the stimulation of the organism by microbial toxins or pathogens [3].

Inflammatory bowel diseases can be acute or chronic. They can be caused by trauma or immune system dysfunction, or of infectious origin, one of the most important being intestinal parasitic infection [4]. Among them, amoebiasis is the third most common cause of death from parasitic infection after malaria and bilharzia [5]. Transmission of this infection occurs through ingestion of the *histolytica* cyst in contaminated food and drink. The symptomatic amoebic infection manifests itself as acute inflammation of the intestine, characterized by episodes of diarrhea with the emission of bloody, mucus-like stools. The acid-resistant *E*. *histolytica* cyst in the stomach during its invasion becomes de-cysted and its nucleus duplicates to give the vegetative form of the amoeba called the trophozoite. The trophozoite releases proteolytic and cytotoxic enzymes that destroy the mucosa and perforate the gut wall [6]. This invasion is in favor of the penetration of the infectious agent into the organism with the recruitment of inflammatory cells, mainly neutrophils and macrophages that produce pro-inflammatory mediators such as Tumor TNF-α, IL-1β, IL-6 thus promoting intestinal proliferation) [7]. Anti-inflammatory cytokines stimulate the secretion of pro-inflammatory cytokines by macrophages which produce the reactive oxygen and nitrogen species (ROS and RNS) responsible for oxidative stress. These ROS and RNS cause damage to biomolecules and their properties.

The drug of choice for the treatment of intestinal amoebiasis is metronidazole. This drug has major side effects such as a metallic taste, headache, dry lips, and colored urine [8]. In most cases, herbal medicine related to the use of plants can impose itself as an alternative, thanks to their richness in secondary metabolites, their availability, their accessibility to all, and presenting lesser side effects. Similarly, according to the [9], more than 80% of the world's population uses medicinal plants to treat various diseases [9, 10]. Thus, knowledge of the biological properties of crude plant extracts, their phytochemicals, and their therapeutic uses for the treatment of certain diseases are of significant importance [11]. *Sida rhombipholia* is a plant in the family *Malvaceae*, class *mangnaliopsida,* and kingdom *plantae*. This seasonal plant is found in tropical and subtropical regions [12]. The plant is known for its wide medicinal use [13] and has antibacterial activities against enteric bacteria [14], antipyretic, cardio-protective [15], and hepato-protective properties [16, 17]. In Cameroon, *S. rhombipholia* is used in traditional medicine in the Noun division (Western region) for the treatment of diarrheic infections and amoebic dysentery. The phytochemical screening of the plant shows the presence of secondary metabolites such as flavonoids, polyphenols, terpenoids, tannins, and phenols [18, 19]. However, the activities of several parts of a plant may differ due to the irregular distribution of secondary metabolites and the variability of extracted compounds, depending on the plant part, harvesting area, and solvent used. The lack of data on this medicinal plant with regards to its anti-amoebic and anti-inflammatory activities prompted us to design this work.

Methods

Plant material

Polyxenic culture of E. histolytica

A biphasic medium of Boeck and Drbohlav [20] that involves a solid phase (ringer's solution + egg) and liquid phase (lock's solution containing nutrients) was used for *E. histolytica* poloyxenic cultivation. Before inoculation, complete media were pre incubated at 37°C for 30 min and 10 µL of polyxenic culture maintained in the Laboratory of Pharmacology and Toxicology of the University of Yaoundé 1 containing the clinical isolates of viable *E. histolytica* trophozoites were added in each tube. The tubes were incubated at 37°C and the *E. histolytica* growth was verified every 48 and 2 h. Then, the tubes were removed from the incubator and shacked to detach parasites from the solid phase and left for 5 min, then the supernatant was decanted to obtain the subculture. The pellet containing the parasites was put in a tube containing pre-incubated new medium and incubated as previously described [21, 22].

Evaluation of amoebic viability

The trypan blue counting method was used. Four plant stock solutions were prepared in sterile DMSO at concentrations 200, 20, 20, and 0.2 mg/mL respectively. Each mixture was filtered with sterile syringe filters (Ø 22 µm) and aliquots were prepared from these stock solutions. Parasites grown were harvested at mid-log phase at the concentration of $1.67x10^7$ cells/mL of culture by counting using the hemocytometer (Neubauer, Hausser Scientific) and inoculated in tubes containing new 5 ml media in which 25 µL of plant materials were added. Metronidazole was used as a standard drug and tested at 1, 10, 50, and 100 µg/mL. *S. rhombifolia* extracts were tested at concentrations of 1; 10; 50; 100, and 500 µg/mL. One control tube containing parasites in 05% DMSO without any drug was used. Each testing concentration was made in triplicate and the experiment was repeated three times. All tested tubes were incubated at 37°C and the viability was evaluated after 24, 48 and 72 h. The amoebicidal activity was assessed using the method previously described [23, 24]. Briefly, in 1.5 mL micro centrifuge tube, 25 µL of parasite suspension and 225 µL of 0.4% trypan blue solution prepared in 0.9% NaCl was introduced. The mixture was homogenized and 10µL of this mixture was used for cell counting. The chamber was covered with a cover slip and the viable (bright) cells as well as the dead (blue) cells were counted at 40X on a light microscope. The concentration of the cell was calculated using the following formula:

$$N= (n\times d)/v$$

Where, N= concentration of viable cells/mL; n= number of the viable cells counted in the chamber, d= dilution factor, and v= the volume of the chamber (0.1 µL). The percentage of inhibition

was calculated using the formula below and IC_{50} was determined using the software Graphpad Prism 3.0

Percentage inhibition (%) = (NC-NT)/NC ×100

NC= Number of viable amoebae in the control tube and NT=Number of viable amoebae in the testing tube

Determination of the anti-inflammatory properties of Sida rhombofolia extracts in vitro

Primary macrophages preparation

Macrophages were isolated and maintained according to the described method [24]. Mice were elicited by an intra-peritoneal injection of 0.5 mL of a 2% starch solution (inflammatory agent). Four days later, animals were sacrificed by cervical dislocation. Then peritoneal primary macrophages obtained using the previously described method [25], were suspended in 1 mL DMEM culture medium and 25µl 2.3×10^7 cells/mL of the suspension were used for trypan blue viability assay [24]. Counted cells were distributed in 96-well microplates at a concentration of 10^4 cells/mL. In the test and positive control wells, 150 µL of cells were introduced with 50 µL of *Saccharomyces cerevisiae* (250 µg/mL). In the blank wells, 150 µL of cells were introduced with 50 µL of DMEM. The micro-plate was incubated for 1h at 37°C (5% CO_2), then 50µL of extract at different concentrations (1, 10, 100, and 500 µg/mL) were added to the test wells and 50 µL of DMEM were added to the positive control wells and finally 50 µL of baicalin to the standard. The microplate was again incubated for 3h at 37°C (5% CO_2). The supernatants were used for the nitric oxide assays, while the pellets were used for the 5-lipoxygenase activity assays and for MTT cytotoxicity.

Cell cytotoxicity assay

The pellets from different incubations were taken up in 100 µL of MTT (3(4,5-dimethylthiazol-2-yl)-2,3diphenyletrazolium) solution (0.5 mg/mL in PBS) and the mixture was incubated at 37°C for 1h 30 min. The supernatant was then removed and 100 µL of acidified isopropanol was added to each tube to dissolve the formazan crystals formed. Finally, the absorbance of the blue-violet solution was read at 550 nm against the acidified isopropanol solution. The percentage of cell viability was calculated using the following formula:

% of cell viability = (sample OD/ control OD)×100

Evaluation of the effect of Sida rhombifolia extracts on nitric oxide (NO) production by viable macrophages

The supernatants previously obtained were transferred to new 96-well microplates. In each well, 100µL of supernatant was mixed with 100 µL of Griess reagent (1% sulphanylamide, 0.1% naphtyl ethylene diamine dihydrochloride in 2.5% v/v phosphoric acid). The mixture was incubated at room temperature for 10 min and the absorbance was measured at 550 nm. The amount of nitrite was determined by reference to the standard calibration curve of the sodium nitrate [26]. The percentage inhibition of NO production was calculated according to the following formula:

% inhibition = ((OD of control- OD of assay)/OD of control) ×100

Evaluation of the effect of S. rhombofolia extracts on 5-lipoxygenase activity

The evaluation of the effect of *S. rhombofolia* extracts was performed in test tubes as previously described [26]. After isolating and recovering the mouse macrophages from the culture medium, 950 µL of cells were introduced into each tube (100000 cells per tube). Then 300 µL of *Saccharomyces cerevisiae* suspension (250 µg/mL) was added to each of the tubes, except the control in which culture medium was added followed by one hour of initial incubation at 37°C (5% CO_2). Subsequently, 50 µL of the extract was introduced in each test tube, 50 µL of ascorbic acid, acetylsalicylic acid, and baicalin in the standard tubes, and finally 50 µL of the medium in control tubes followed by three hours of second incubation. Each tube was centrifuged at 2000 rpm for 10 minutes at 4°C and the supernatant was discarded. In pellet-containing cells, 50 µL of Triton X-100 was added and the tubes were shaken for 2 minutes then 1000 µL of linoleic acid (125 µM) was added and the mixture was incubated once more for 30 minutes. Absorbance was read at 234nm. The inhibition percentage of enzyme activity was calculated according to the following formula:

% Inhibition = ((OD of control – OD of test)/ OD of control) ×100

Determination of Total phenolic and flavonoid compound contents of S. rhombifolia extracts

Determination of total phenolic compound contents

In each tube, 2000 µL of distilled water, 100 µL of different extracts at 100 µg/mL, and 200µL of Folin-ciocalteu (2N) solution were introduced. The calibration curve of gallic acid was done at a fixed concentration of 100µg/mL at different volumes (0, 20, 40, 60, 80, 100 µL) and by completing the volumes to 100µL with pure methanol and then 200µL of Folin-ciocalteu was also added. The mixture was allowed to stand for 3 min, then we added 1000 µl of 20% sodium carbonate. Then the mixture was incubated for 1 hour in the dark at room temperature. The absorbance was read at 700 nm. The total phenolic compound contents were expressed as milligram equivalents of gallic acid per gram of extract (mg GAE/g extract) using the calibration line [27].

Determination of flavonoid contents

In the blank and test tubes were respectively introduced: 500 µL of methanol, 500 µL of different extracts, and 500 µL of aluminum trichloride ($AlCl_3$) 2% (m/v). The mixtures obtained were homogenized using the vortex and the tubes were then incubated at room temperature for 1 h. The optical densities of the yellow coloration were read at 430 nm against the blank. The assay was performed in triplicate and Quercetin was used as a standard. Flavonoid contents are expressed as milligram equivalents of Quercetin per gram of extract (mg EQ/g extract). Using the calibration line [27].

Statistical analysis

Statistical analyses of the values obtained were carried out using Graphpad Prism 8.0.1 software and Excel spreadsheet. The results were expressed as mean ± standard deviation and the different values were compared using the analysis of variance test "one-way ANOVA" as well as the Turkey multiple comparison test and the differences were considered significant for a p-value $p<0.05$.

Results

Anti-amoebic potential of different extracts of S. rhombifolia

The clinical isolates of *E. histolytica* maintained on the biphasic medium of Boeck and Drbohlav (Figure 1) were incubated with different plant extracts. The variation of trophozoite number as a function of concentration and incubation time observed with the optical microscope shows a significant decrease in the number of parasites (Figure 2). From this figure, it can be observed subsequently that, after 24h, 48, and 72 h post-treatment, there was a significant reduction of amoebic viability in the tested tubes as compared to the control tubes. The amoebicidal activity of *S. rhombifolia* extracts is represented by the percentage of parasite viability, at different incubation periods and at different concentrations of plant extracts and compounds (Figure 3). It was observed that the amoebicidal activities of the extracts and metronidazole were concentration-dependent. From these amoebicidal activities, inhibitory concentrations fifty (IC_{50}) were determined (Table 1) and it was found that the harvesting area hadn't affected the amoebicidal activities of ethanolic leaf extract after 72 h. However, ethanolic extracts were found to be more active than aqueous extracts after 72h. Those activities remained lower compared to metronidazole (the reference drug).

Anti-inflammatory activity of S. rhombifolia aqueous and ethanolic extracts

Cytotoxicity of extracts on primary macrophage culture

The evaluation of the cytotoxicity of *S. rhombifolia* extracts on primary macrophages was carried out using MTT assay at different extract concentrations, (Figure 4). The results revealed that no significant decrease was observed in cell viability in the presence of extracts at 500 µg/mL, between zero and six hours.

Effect of the extracts on the production of nitrite oxide (N.O)

S. rhombifolia extracts effectively inhibit the production of nitrite oxide and the inhibitory effect was concentration-dependent (Figure 5a). The determination of IC50's (Table 2) demonstrated that no significant difference was observed between the inhibitory effects of all the ethanolic extracts, all the aqueous leaf extracts, and baicalin (standard). However, the inhibitory effects of all the stem extracts were significantly the lowest ($p<0.05$).

Effect of the extracts on 5-lipoxygenase activity in activated macrophages

The inhibitory activity of the extracts on the 5-lipoxygenase of activated mouse macrophages was evaluated by hydroperoxide assay. It was observed that there was a concentration-dependent decrease in the activity of this enzyme in the presence of the plant extract (Figure 5b). IC_{50} obtained (Table 2) revealed that only leaf ethanolic extracts (LEEF and LEEM) exhibited potent inhibitory activity even though less active than the reference compound. (Respectively 36.64 ± 9.61 µg/mL for LEEY, 73.22 ± 37.43 µg/mL for LEEM, and 11.93 ± 3.93 µg/mL for Baicalin). However lowest and neglected activities were observed with other extracts.

Total phenolic compounds and flavonoids of aqueous and ethanolic extracts of S. rhombifolia

As phenolic compounds are compounds with a wide range of biological activities, their levels were evaluated in the different plant extracts (Table 2). SEEY was the extract presenting the highest level of total phenolic compounds and flavonoids (respectively 64.27 ± 1.88 mgEGA/g of extract for total phenolic compound and 6.57 ± 0.01 mgEQ/g of extract for flavonoid content).

Discussion

Entamoeba histolytica is an intestinal parasite that has a high mortality and morbidity rate in developing countries [5]. Many studies have been conducted to improve the assistance given to infected patients in the hospital. Some others are still to be done; and some of them are using a polyxenic culture of the parasite to mimic their living environment in the intestine [21, 22]. To the best of our knowledge, this study is the first report on the antiamoebic properties of crude extracts from *S. rhombifolia*. In the present work, the amoebicidal activity of extracts of different parts of *S. rhombifolia* was evaluated on clinical isolates of *E. histolytica* in polyxenic culture. From the results obtained, it appears that the ethanolic extract of *S. rhombifolia* leaves, independently of their harvesting area, is more active on *E. histolytica* trophozoites in polyxenic culture after 72h of incubation. This observation could be explained by the fact that the activities of several parts of a plant may differ due to the variability of extracted compounds, depending on the solvent used. Based on previous work an extract with a CI_{50} ≤ 10µg/ml is active [28]. This activity was significantly not different from that of metronidazole. These results were obtained with the aqueous leaf extract of *C. variegatum* with the inhibitory concentration 50-fold less than 15 µg/mL against clinical isolates in polygenic culture [21]. The results are also close to those obtained with the same extract against the HM1: IMSS strain in axenic culture [8]. However, the activities observed with the ethanolic crude extract of *S. rhombifolia* are higher as compared to those obtained with the aqueous and methanolic extract of *E. hirta* against clinical isolates of *E. histolytica* on polyxenic culture [22] and crude methanol extract of *A. mexicana* against the same strain [29]. During *E. histolytica* infection interaction of the pathogen with intestinal epithelial cells throughout the lectine Gal/NAc- TLR2/4 binding triggers the stimulation of immune cells system within the host cell characterized by an increased level of pro-inflammatory mediators [30]. A shift in the balance between the pro and anti-inflammatory mediators may lead to inflammation.

Macrophages have a central and essential role in the inflammatory process through their surface membrane receptors (TLRs) [31]. They produce numerous pro- and anti-inflammatory mediators among which NO responsible for the dilatation of blood vessels, leukotrienes B4 (LTB4) that acts as a chemoattractant molecule synthesized by 5-lipoxygenase. However, an imbalance between the pro-inflammatory and anti-inflammatory systems in

favor of the pro-inflammatory system would be the cause of pathologies such as inflammatory diseases. The determination of the anti-inflammatory activity of *S. rhombifolia* extracts was carried out by inhibiting those pro-inflammatory actors. Before carrying out those activities the cytotoxicity of *S. rhombifolia* extracts was determine by the MTT assay which focusses on the activity of succinate deshydrogenase of those cells. The results obtained from the MTT test in the presence of different extracts show no significant decrease in cell viability at the concentrations tested. These results indicate the cellular activity of the extracts on cell viability and are in agreement with those obtained in previous findings [32] through the study of aqueous and ethanolic extracts of *Globularia alypun* and *Hertia cherifolia* on macrophages isolated from mice and activated by LPS which did not show cytotoxic effects on these cells in culture. Similarly, the results are better than those obtained by Léa et al., [33], who showed that *Sealapex spress* extracts had cytotoxic effects on activated macrophages at a concentration of 10µg/mL. Nitric oxide is a pro-inflammatory mediator produced by specialized cells of the immune system [34]. Nitric oxide stimulates vasodilation, increased membrane permeability, and recruitment of other inflammatory cells. However, at high levels of NO, it has adverse effects on the body's cells ranging from cytotoxicity to cell damage leading to mutagenesis. The inhibition of its synthesis is therefore a sign of anti-inflammatory activity. The results obtained showed that, ethanolic extracts of the leaves independently of their harvesting areas exhibited a significant inhibitory effect on the production of NO by *Saccharomyces cereviseae* activated macrophages as compared to that of baicalin (reference compound). In contrast to the ethanolic extracts, the aqueous extracts have a very low anti-inflammatory activity with a significantly lower inhibitory effect than baicalin. The values found for the aqueous extracts are lower compared to those previously found [35] with the aqueous extracts of lentinan. 5-lipoxygenase is an enzyme derived from arachidonic acid metabolism and is responsible for the synthesis of the pro-inflammatory mediator leukotrienes (LTB4) [36]. 5-lipoxygenase also plays a central role in regulating the interaction between innate and adaptive immunity. Investigation of the effect of the extracts on 5-lipoxygenase activity showed that ethanolic leaf extracts independently of their harvesting locations significantly inhibited the activity of this enzyme (CI_{50} = 36.64±9.61 µg/mL; 73.22±37.43 µg/mL respectively), although lower than that of baicalin (reference compound) (CI_{50} = 11.93±3.93 µg/mL). These results demonstrated that the extracts might inhibit immune cell mobility at the inflammation site and might also antagonize metabolic interactions. These results are like those obtained by [27] who were able to show a significant decrease in 5-lipoxygenase activity in the presence of *Saba senegalensis* extracts. However, the phytochemical analysis test suggests that the ethanolic extract presented high phenolic compound content than aqueous extracts. Previously published results [36, 25] showed the presence of similar secondary metabolites in the ethanolic extract of *Codiaeum variegatum* flowers and stem.

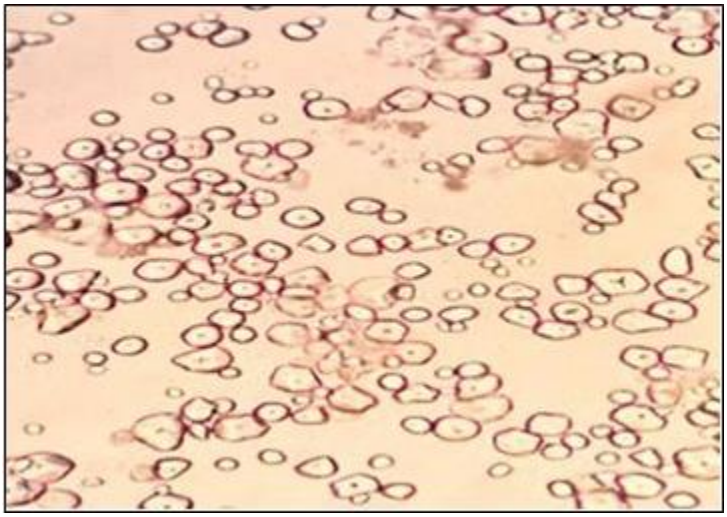

Figure 1. *E. histolytica* trophozoites in polyxenic culture observed under the light microscope at 40X objective before treatment with plant extracts.

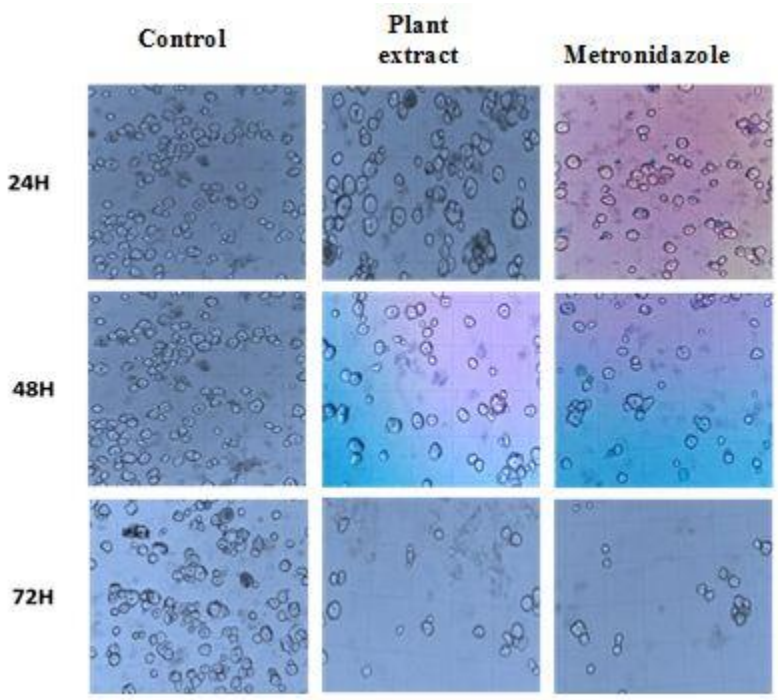

Figure 2. Microscopic observation (40X objective) showing the variation in the number of *E. histolytica* trophozoites after treatment with plant extracts and compound as a function of time.

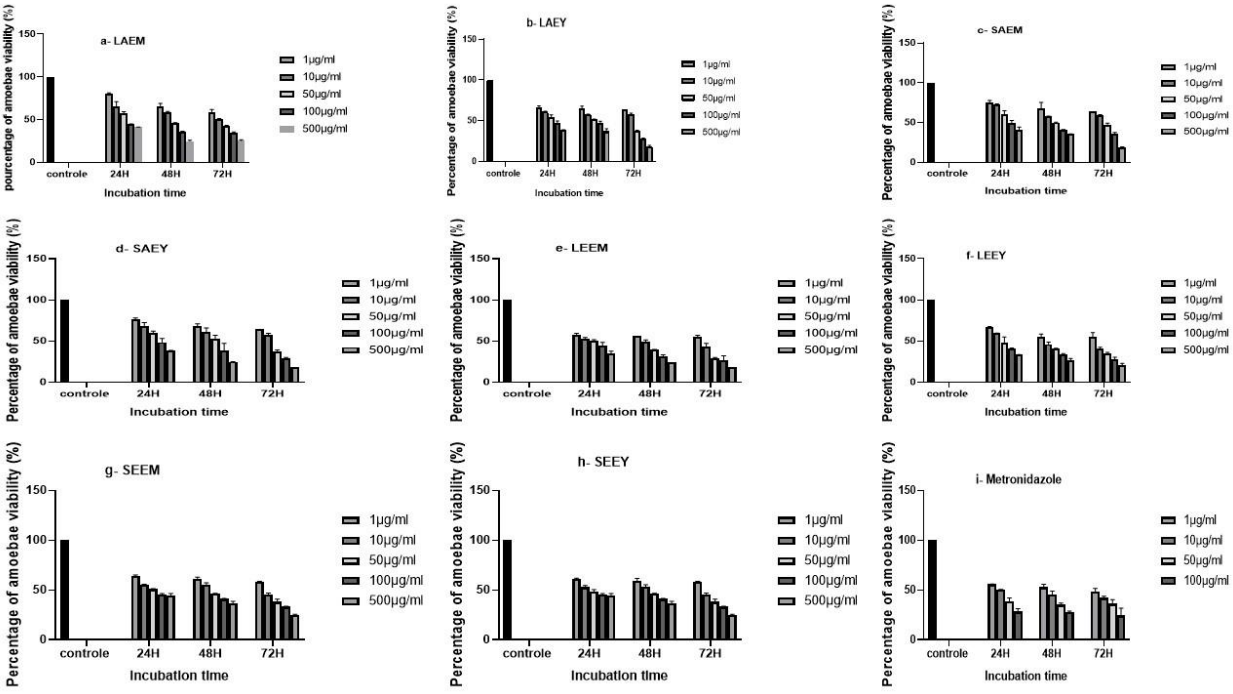

Figure 3. Amoebicidal activity of *S. rhombifolia* extracts and Metronidazole against clinical isolates of *Entamoeba histolytica* on polyxenic culture.

LAEM: *leave aqueous extract from Massangam,* ***LAEY:*** *leave aqueous extract from Yaoundé;* ***SAEM:*** *stem aqueous extract from Massangam;* ***SAEY :*** *stem aqueous extract from Yaoundé;* ***LEEM:*** *leave ethanolic extract from Massangam ;* ***LEEY:*** *leave ethanolic extract from Yaoundé;* ***SEEM:*** *stem ethanolic extract from Massangam*; ***SEEY:*** *stem ethanolic extract from Yaoundé;*

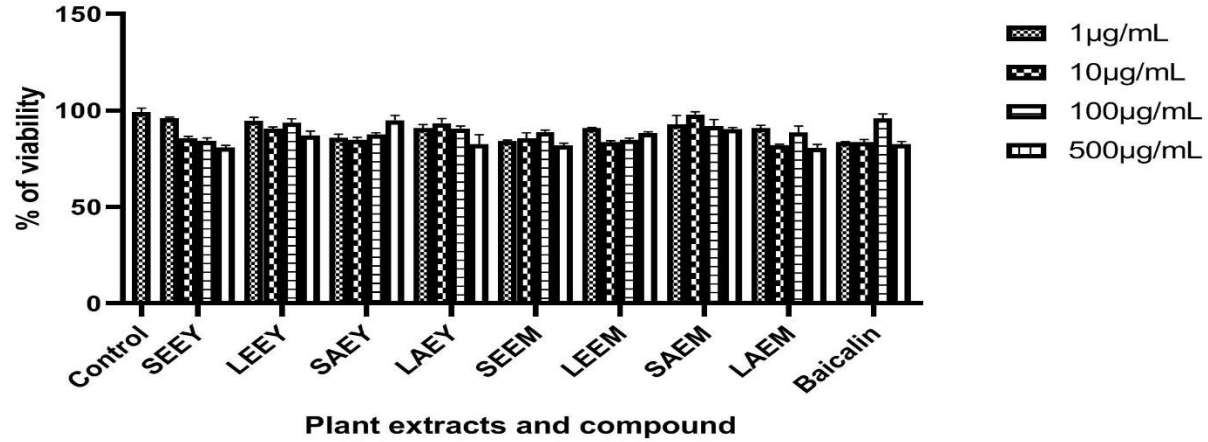

Figure 4. Effect of *S. rhombifolia* extracts and compound on the growth of primary isolated mouse macrophage.

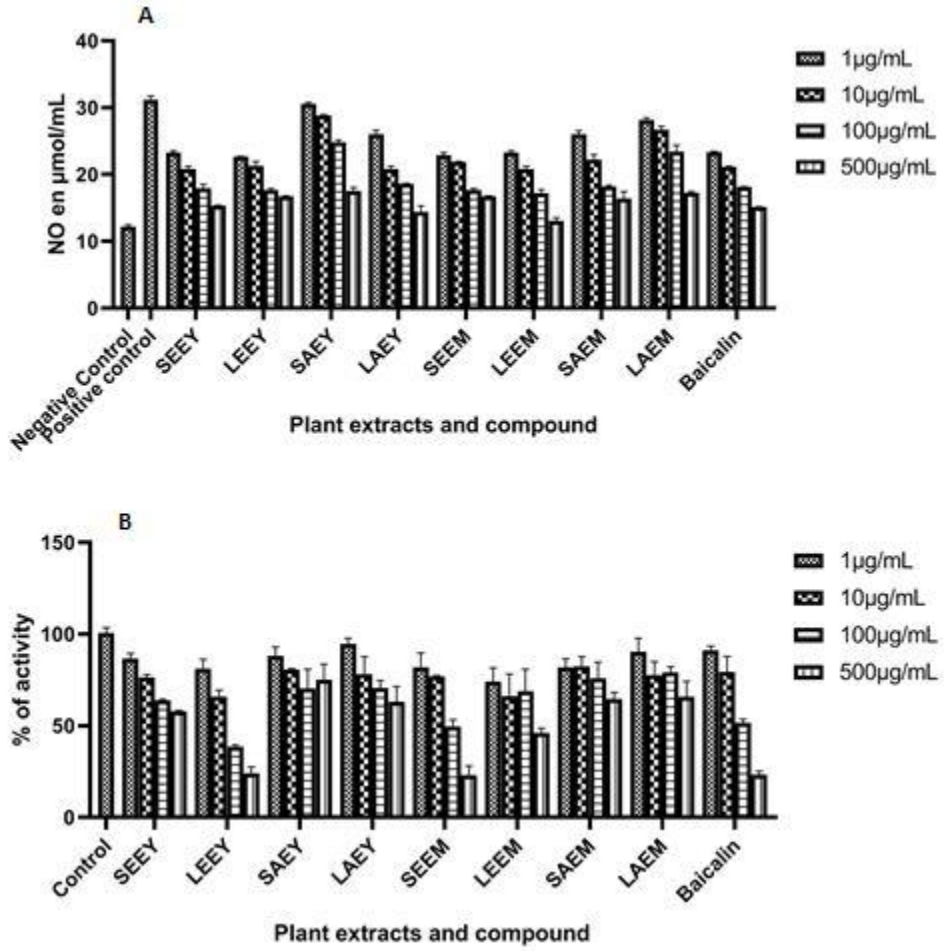

Figure 5. Anti-inflammatory activity of *Sida rhombifolia* extracts.

5a. Inhibitory effect of *S. rhombifolia* extracts and compound on NO production; **5b.** Inhibitory effect of *S. rhombifolia* extracts and compound on 5-Lipoxigenase activity.

SEEY: *stem ethanolic extract from Yaoundé; LEEY: leave ethanolic extract from Yaoundé;* ***SAEY:*** *stem aqueous extract from Yaoundé;* ***LAEY:*** *leave aqueous extract from Yaoundé ;* ***SEEM:*** *stem ethanolic extract from Massangam*; ***LEEM:*** *leave ethanolic extract from Massangam ;* ***SAEM:*** *stem aqueous extract from Massangam;* ***LAEM:*** *leave aqueous extract from Massangam ,* ***MTZ****: metronidazole.*

Table 1. Inhibitory concentration 50 of *S. rhombifolia extracts* and metronidazole on *Entamoeba histolytica* viability within time

Samples	Incubation time and IC_{50} (µg/mL)		
	24 h	**48 h**	**72 h**
SSEY	52.35 ± 4.77	38.59 ± 9.55	12.82 ± 0.85
SEEM	59.78 ± 2.59	40.71 ± 4.53	15.08 ± 0.77
LEEY	46.21 ± 8.54	17.47 ± 4.47	7.71 ± 0.54*
LEEM	47.62 ± 1.16	18.04 ± 1.22	7.77 ± 0.58*
SAEY	89.76 ± 8.02	46.85 ± 4.77	24.78 ± 0.58
SAEM	96.12 ± 2.62	47.94 ± 4.68	34.77 ± 1.77
LAEY	67.61 ± 5.82	60.17 ± 5.85	18.64 ± 3.04
LAEM	74.74 ± 4.003	34.83 ± 13.94	21.12 ± 1.96
Metronidazole	15.04 ± 1.22	9.77 ± 1.02	5.96 ± 0.58

SEEY: *stem ethanolic extract from Yaoundé; LEEY**:** leave ethanolic extract from Yaoundé;* ***SAEY :*** *stem aqueous extract from Yaoundé;* ***LAEY:*** *leave aqueous extract from Yaoundé ;* ***SEEM:*** *stem ethanolic extract from Massangam*; ***LEEM:*** *leave ethanolic extract from Massangam ;* ***SAEM:*** *stem aqueous extract from Massangam*

Table 2. Inhibitory concentration 50 of plant extracts and compound for anti-inflammatory assays and total phenolic compounds and flavonoids contents

Parameters	Samples								
	SSEY	**SEEM**	**LEEY**	**LEEM**	**SAEY**	**SAEM**	**LAEY**	**LAEM**	Baicalin
IC_{50} of NO inhibition (µg/mL)	4.43 ± 0.57*	5.10 ± 0.81*	5.85 ± 0.88	3.99 ± 12.45	194.5 ± 12.45	121.4 ± 11.41	9.89 ± 1.52	14.98 ± 4.39	4.97 ± 1.96
IC_{50} of 5-Lox inhibition (µg/mL)	>500	>500	36.64 ± 3.61	73.22 ± 7.43	ND	ND	>500	>500	11.93 ± 3.93
Total phenolic compounds contents (m EGA/g)	24.88 ± 0.88	21.85 ± 0.17	64.27 ± 1.88	41.08 ± 0.17	34.49 ± 0.96	30.36 ± 1.88	27.15 ± 0.10	15.79 ± 0.2	
Total flavonoids contents (m EQ/g)	4.32 ± 0.01	4.38 ± 0.10	6.57 ± 0.01	4.38 ± 0.00	1.32 ± 0.01	1.40 ± 0.00	3.09 ± 0.00	3.30 ± 0.00	

SEEY: *stem ethanolic extract from Yaoundé; LEEY**:** leave ethanolic extract from Yaoundé;* ***SAEY:*** *stem aqueous extract from Yaoundé;* ***LAEY:*** *leave aqueous extract from Yaoundé ;* ***SEEM:*** *stem ethanolic extract from Massangam*; ***LEEM:*** *leave ethanolic extract from Massangam ;* ***SAEM:*** *stem aqueous extract from Massangam* ***LAEM:*** *leave aqueous extract from Massangam .*

Conclusion

At the end of this work, it can be concluded that the ethanolic leaf extracts of *S. rhombifolia*, regardless of the harvesting area, exhibited the best anti-amoebic potentials on a polyxenic cultures of *E. histolytica* and the best anti-inflammatory potentials on primary culture of peritoneal macrophages.

Abbreviations

DMEM: Dulbecco's Modified Eagle Medium
IC_{50}: Inhibitory concentration 50
LPS: Lipopolysaccharide
LAEM: leave aqueous extract from Massangam
LAEY: leave aqueous extract from Yaoundé
SAEM: stem aqueous extract from Massangam
SAEY: stem aqueous extract from Yaoundé
LEEM: leave ethanolic extract from Massangam
LEEY: leave ethanolic extract from Yaoundé
SEEM: stem ethanolic extract from Massangam
SEEY: stem ethanolic extract from Yaoundé
MTT: 3(4,5-dimethylthiazol-2-yl)-2,3diphenyletrazolium
LTB4: Leucothrien B4
NO: Nitric Oxide

Authors' Contribution

SPN, RNN, BEE and EMN carried out all experiments reported in the manuscript. SPN, FNN and PFM designed the study. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest

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