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Hydro-Alcoholic Leaf Extract and Fractions of *Codiaeum variegatum* (var. Mollucanum) Exhibited an Improved Anti-Amoebic and Moderate Anti-Oxidant Potential

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Abstract

Amoebiasis, classified as the third intestinal parasitic infection, represents a public health problem in low-income countries where hygiene and sanitation conditions are poor. With the resurgence of resistant pathogenic strains as well as ancestral considerations in developing countries such as Cameroon, many people rely on medicinal plants to treat a plethora of diseases. This work aimed to highlight the anti-amoebic and anti-oxidant potential of *Codiaeum variegatum* extracts and fractions. The anti-amoebic potential of *C. variegatum* was assayed on the polyxenic culture of the clinical isolates of *E. histolytica*. Then, the anti-oxidant potential of the ethanolic/hydroethanolic extracts and fractions was evaluated through DPPH radical scavenging, iron reduction (FRAP), lipid peroxidation inhibitory potential and total antioxidant capacity tests followed by the determination of phenolic compound and flavonoid content. It was found that the fractionation process decreased the amoebicidal activities of *C. variegatum* leaf extracts. However hydroethanolic extract (CI_{50}: 10.08 ± 0.42, 5.18 ± 0.09, 5.18 ± 0.09 µg/mL respectively after 24, 48 and 72 hours) was more active than ethanolic extract (CI_{50}: 15.59 ± 6.17; 9.61 ± 2.37; 6.26 ± 3.22 µg/mL respectively after 24, 48 and 72 hours). Interestingly, the activities of hydroethanolic extract were significantly non-different compared to metronidazole CI_{50}: 8.42 ± 0.44, 6.45 ± 0.22 and 3.42 ± 0.33 µg/mL, respectively after 24, 48 and 72 hours). Ethanolic extract and EF5 showed higher Phenolic compound contents and higher antioxidant activity

than hydroethanolic extract and other fractions through DPPH radical scavenging power (EC50 = 311.50 ± 4.12 µg/mL) and total antioxidant capacity (44 ± 0.07 mgEAA/gF). However, these activities are significantly lower than those of ascorbic acid (EC50 = 31.20 ± 4.39 µg/mL, and 61.34 ± 4.42 µg/mL respectively). This low antioxidant activity was confirmed by poor phenolic and flavonoid compounds contents found in the extracts and fractions. The present result brings a new approach to the ethnopharmacological uses of *C. variegatum* against dysentery in cases associated with Amoebiasis in Cameroun.

Keywords

Codiaeum Variegatum, Amoebiasis, Antiamoebic, Antioxidant

1. Background

Entamoeba histolytica infection is ranked as the third leading intestinal parasitic cause of human mortality worldwide [1] [2]. Approximately 50 million people worldwide suffer from invasive amoebic infection and account for 10 million cases of dysentery each year, resulting in 40 - 100 thousand deaths annually [3], 10% of the population worldwide suffer from this disease. In Cameroon, the percentage of infection varies between regions and the prevalence of this infection was reported to be 28.7% in patients infected with HIV in this country [4] [5]. *E. histolytica* exists in two forms the cyst and trophozoite. The cyst form lives silently in the human colonic mucin and is transformed into a trophozoïte invasive form. In the colonic epithelium, the invasive form of amoeba overcomes innate host defence through secretion of lytic cysteine proteinase that degrades colonic mucin in the intestinal mucus (first line of host defense against invasive pathogens), alters its function, and leads to amoebic dysentery [6]. This invasion takes place by contact between the Gal/GalNac lectin present on the surface of the trophozoite of *E. histolytica* through TLR-2/4 of epithelial cells and triggers a host immune response characterized by increased levels of pro-inflammatory mediators and pro-oxidants that recruit inflammatory cells [7]. These pro-oxidants such as peroxide radicals (O_2), hydroxyl radicals (OH·), peroxide (H_2O_2), hydroperoxide and nitric oxide radicals (NO) produced by the inflammatory cell of the host are directed against the invading parasites [8]. Those invading *E. histolytica* can protect themselves from reactive oxygen species (ROS), and reactive nitrogen species (RNS) with peroxiredoxin, a potent parasitic anti-oxidant [9] [10], by triggering the production of pro-inflammatory cytokines namely Tumor Necrosis Factor Alpha (TNF-α), interferon gamma and interleukin-1beta (IL-1β) [11]. Prolonged hypersecretion of these free radicals could lead to an imbalance in favor of pro-oxidants resulting in a pathological state characterized by the oxidation of biomolecules altering their functions [12]. In absence of treatment, trophozoite passes via the blood circulatory system to invade other organs like

the liver, brain and lung. Chemotherapy has been used for treating amoebiasis and Nitroimidazole (diloxanide furoate, iodoquinol, metronidazole, chloroquine, iodoquinol) are drugs used for this treatment [13]. Metronidazole, the common drug of choice used has been reported to be genotoxic to human cells and is associated with side effects like constipation, vision problems, stomach cramps, loss of appetite, eye pain, skin itching, and vomiting [14]. The drug may also be immune suppressive [15]. Medicinal plants have long been recognised as alternatives in the treatment of various diseases, because they contain bio-active compounds [16]. *C. variegatum* has been widely investigated, and has been reported to exhibit multiple medicinal properties. The aqueous extract of *C. variegatum* was active against both polyxenic and axenic culture *E. histolytica* [17] [18], exhibited anticonvulsant [19], anti-giardial and antitrichomonal potential [20], antioxidant and anti-inflammatory [21] [22] [23], and hence do not present any sub-chronic toxicity [24]. However, the variability of extracted active secondary metabolites from plant parts may differ due depending on the solvent used. Although many studies have already reported anti-amoebic activity on aqueous extract of *C. variegatum*, no finding has yet corroborated this activity with its hydroethanolic leaf extract and fractions. Therefore, the present study aimed to investigate the antioxidant and antiamoebic potentials of hydro-alcoholic extract and fractions of *C. variegatum*.

2. Methods

2.1. Plant Material

Leaves of *C. variegatum* (var. mollucanum) were collected in the locality of Nomayos, in the Centre region of Cameroon. The specimen was identified under number HNC 33570 at the Cameroon National Herbarium (CNH) in Yaounde, Cameroon. The stems were washed and rinsed with distilled water and dried at laboratory temperature, then crushed in a blender to obtain the powder and preserved. The resulting powders were packaged and stored at 4˚C for later use.

2.1.1. Preparation of the Plant Extracts

Powdered plant material (400 g) was macerated in 4 l of ethanol/water in the ratio (70:30 v/v) or ethanol (95% v/v) for 48 hours at room temperature. The resulting extract was filtered through a whatman N˚1 filter paper and then dried using a rotary evaporator at 65˚C. The residues which constitute the crude extract were kept at 4˚C until further use.

2.1.2. Fractionation of the Leaf Extracts of Codiaeum Variegatum

Fractionation was done by flash chromatography using several solvents or mixture of solvents in the following order: methylene chloride, methylene chloride/methanol (95:5 v/v), methylene chloride/methanol (90:10 v/v), methylene chloride/methanol (50:50 v/v) and methanol (**Figure 1**). The solvent change was made when the filtrate appeared clear. At the end of the procedure, all filtrates obtained with the same solvent were mixed and then concentrated in a rotary

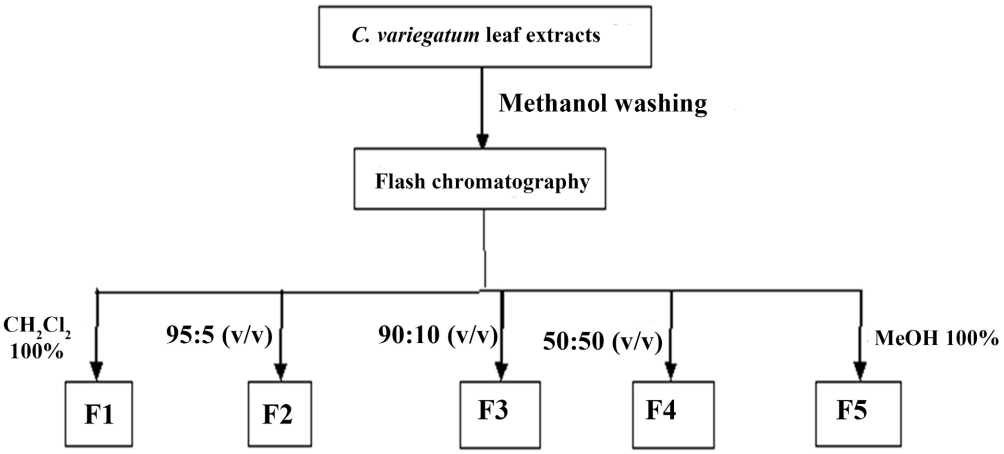

Figure 1. Fractionation process of *C. variegatum* leaf extracts by flash chromatography.

evaporator. The final fraction obtained was kept in a clean bottle and stored at 4˚C. Each fractionation yield (FY) was calculated according to the formula below.

Fractionation yield = (masse of fraction/masse of extract) × 100

2.2. Biological Material

The biological animal material used consisted of macrophages prepared from mice and clinical isolates of *E. histolytica* maintained on a polyxenic culture medium at the Laboratory of Pharmacology and Toxicology of the University of Yaounde 1.

2.3. Evaluation of the Anti-Amoebic Properties of the Aqueous and Ethanolic Extract of the Leaves and Stems of *S. rhombifolia* in Polyxenic Culture

2.3.1. Polyxenic Culture of *E. histolytica*

Biphasic medium of Boeck and Drbohlav [25] that involves 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 to 1 h and 10 µL of polyxenic culture maintained in the Laboratory of Pharmacology and Toxicology of the University of Yaounde1 containing the clinical isolates of viable *E. histolytica* trophozoites *were* introduced in each tube. The tubes were incubated at 37˚C and the *E. histolytica* growth verified after every 48 or 72 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 introduced in a tube containing pre incubated new medium as previously described [18] [26].

2.3.2. Test of Amoebic Viability by Trypan Blue Counting Method on *E. histolytica* Polyxenic Culture Medium

S. rhombifolia aqueous and ethanolic extracts were prepared using sterile DMSO

(Sigma-Aldrich, and liquid phase of culture medium leading to concentrations of 200, 20, 2, 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 midlog phase at the concentration of 1.67×10^7 cells/ml of culture by counting using the haemocytometer (Neubauer, Hausser Scientific) and inoculated in tubes containing new 5 ml media in which 25 µl of plant materials were added. MTZ was used as a standard drug and was tested at 1, 10, 50 and 100 µg/mL. *S. rhombifolia* extracts were tested at the concentration of 1; 10; 50; 100 and 500 µg/mL µg/ml. One control tube was used in which parasites were incubated on culture medium containing 0.5% DMSO without any drug. Each testing concentration was made in triplicate and the experiment was repeated three times for each compound. All the tested tubes were incubated at 37˚C as previously described [27] and the viability was evaluated by trypan blue method after 24, 48 and 72 h. Amoebicidal activity was evaluated using the method described by [28]. 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 10 µl of this mixture was used for cells counting. The chamber was covered with cover slip and the viable (bright) cells as well as the dead (blue) cells were counted at 40× on a light microscope. The concentration of the cell has been 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 percentages of inhibition were calculated also using the formula below and IC50 were determined using the software Graphpad Prism 3.0,

$$\text{Percentage inhibition (\%)} = (NC - NT)/NC \times 100$$

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

2.4. Evaluation of the Antioxidant Property of *Codiaeum variegatum*

The antioxidant property of *C. variegatum* was evaluated by using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging, lipid peroxidation inhibition, ferric iron reducing power assays and by determining the total antioxidant capacity. For these different tests 50 µL of extracts at concentrations of 1; 10; 100; 500 and 1000 µg/mL were used.

2.4.1. DPPH Radical Scavenging Assay

This assay was carried out following a method previously described [29]. Briefly in a series of test tubes containing 3.1 mL of the methanolic solution of DPPH (40 µg/mL), 50 µL of plant extracts at different concentration was added. In the negative control tubes, the extract was replaced by 50 µL of solvent and the posi-

tive control by 50 µL of ascorbic acid. The mixtures were homogenized and incubated in the dark for 30 minutes at room temperature, and the absorbance was measured at 517 nm with a spectrophotometer. The percentages of inhibition were calculated by using the following formula:

$$\% \text{ of DPPH scavenging activity} = [(OD_{control} - OD_{assay})/OD_{control}] \times 100$$

where: $OD_{control}$: absorbance of the negative control tube;

OD_{assay}: absorbance of the test tube.

The IC50 value expressed in µg of extract per mol of DPPH for each extract was determined by using a non-linear regression curve of the DPPH scavenging activity against the concentration of extracts tested.

2.4.2. Evaluation of the Inhibition of lipid Peroxidation

1) Preparation of the liver homogenate

One Wistar rat was sacrificed by cervical dislocation and the liver was excised. The rest of the manipulation was done in ice. The organ was washed in a saline solution of 0.9% NaCl and then spin-dried and weighed. Once the mass of the liver was known, it was cut into small pieces in a solution of 1.15% KCl and crushed using the Teflon plunger of the Potter apparatus and Bleau fountain sand. A 10% homogenate in a 1.15% KCl solution was then prepared taking into account the weight of the liver, divided into several tubes and centrifuged (720 g, 10 min, 4˚C). Each supernatant was collected and the volume noted. Depending on the number of assays to be performed, aliquots were prepared and stored in the freezer until use.

2) Lipid peroxidation inhibition assay

Thiobarbituric acid reactive substances were determined by using previously described method [30]. In each test tube, 50 µL of plant extracts, 1 mL of 10% rat liver homogenate, 50 µL of 0.5 mM $FeCl_2$ and 50 µL of 0.5 mM H_2O_2 were successively introduced. In the blank tube, $FeCl_2$ and H_2O_2 were replaced by 100 µL 1.15% KCl while in the negative control tube, the extraction solvent was used instead of the extract. The mixtures were incubated (1 hour, 37˚C). After incubation, 1mL trichloroacetic acid (TCA 15%) and 1 mL 0.67% TBA were added to all tubes and boiled in a water bath for 15 minutes. After cooling and centrifugation (1620 g, 5 min, 4˚C), the supernatants were collected and the absorbance of the pink stain read at 532 nm against the blank. The percentages of inhibition were calculated using the formula below.

$$\% \text{ of lipid peroxidation inhibition} = [(OD_{control} - OD_{assay})/OD_{control}] \times 100$$

where: $OD_{control}$: absorbance of the negative control tube;

OD_{assay}: absorbance of the test tube.

The evolution of the percentage of inhibition according to the concentration of extract used allowed us to determine the IC_{50} in µg/mL.

2.4.3. Ferric Reducing Power Assay

The ferric reduction power assay was performed as previously described [31]. In each tube 50 µL of plant extracts, 1100 µL of phosphate buffer (0.6 M pH 6.6),

1000 µL of 0.25% potassium ferricyanide were introduced. In the blank, 1100 µL of distilled water was added instead of potassium ferricyanide. After incubation for 20 min at 50˚C, 1 mL of 10% trichloroacetic acid (TCA) was added to all tubes. The whole set was centrifuged (1620 g, 10 min 4˚C) then, to 1mL of supernatant were added 1mL of distilled water and 200 µL of ferric chloride. The whole was well homogenized and then left to stand for 10 min. The absorbance was measured at 700 nm against the blank in a spectrophotometer and the percentages of iron reduction were calculated by the following formula:

$$\% \text{ reduction} = [(OD_{control} - OD_{assay})/OD_{control}] \times 100$$

where: $OD_{control}$: absorbance of the negative control tube;

OD_{assay}: absorbance of the test tube.

The evolution of the percentage of reduction as a function of the extract concentration allowed us to determine the EC_{50}.

2.4.4. Evaluation of the Total Antioxidant Capacity

The TAC was measure following the method previously described [32]. In each test tube 50 µL of plant extracts, 1 mL of 0.6 M sulphuric acid, 1050 µL of 28 mM sodium phosphate and 1050 µL of 4 mM ammonium molybdate were successively introduced. The tubes were capped with the beads and heated for 90 min then cooled on a stream of cold water. The absorbance of the blue staining mixture was measured in a spectrophotometer at 695 nm. The antioxidant capacity of the extracts expressed in g ascorbic acid/mg extract was determined from the calibration curve obtained by using different concentrations of ascorbic acid instead of plant extracts.

2.4.5. Determination of Total Phenolic Compound and Total Flavonoid Contents

The total phenolic and total flavonoid contents of different extracts and fractions were determined using the modified Folin-Ciocalteu method [20] [33]. Gallic acid and Quercetin were use as standard respectivly.

2.5. Data Analysis

Data analyses were performed using GraphPad Prism 8.0.1 software. The results were expressed as mean ± standard deviation and the different values were compared using the analysis of variance test "one-way ANOVA" followed by the multiple comparison test of Turkey with a p-value $p < 0.05$.

3. Results

3.1. Extraction and Fractionation Yields

Ethanolic extraction yield was higher than hydroethanolic extraction yield (20.5% and 16.5% respectively. Amoung fractions, EEF5 exhibited the highest fractionation yield (57.9%), whereas EEF3 exhibited the lowest fractionation yield (0.8%). (**Table 1**)

Table 1. Extraction and fractionation yield of *C. variegatum*.

Extracts/Fractions	HE	EE	HEF1	HEF2	HEF3	HEF4	HEF5	EEF1	EEF2	EEF3	EEF4	EEF5
Fractionation yields	16.50%	20.50%	3.54%	3.54%	9.60%	18.70%	24.80%	29.90%	0.80%	0.80%	2.40%	57.90%

3.2. Anti-Amoebic Potential of Leaf Extracts and Fractions of *Codiaeum variegatum on* Polyxenic Culture of Clinical Isolate of *E. histolytica* Trophozoites

The clinical isolates of *E. histolytica* maintained on biphasic medium of Boeck and Drbohlav were incubated with different plant extracts and fractions. The variation of trophozoites number as a function of concentration at different incubation times observed with the optical microscope showed a significant decrease in number of the parasites (Figure 2). From this figure, it can be observed that subsequently, after 24 h, 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 leaf extracts and fractions of *C. variegatum* are represented by the percentage of parasite viability, assessed by the trypan blue counting method at different incubation periods and at different concentrations of plant extracts, fractions and metronidazole (Figure 3). As results, it was observed that the amoebicidal activities of the extracts, fractions and metronidazole were concentration-dependent. From these amoebicidal activities, inhibitory concentrations fifty (IC_{50}) were determined (Table 2) and it was found that fractionation process decreased the amoebicidal activities of *C. variegatum* leaf extract. However hydroethanolic extract (CI_{50}: 10.08 ± 0.42, 5.18 ± 0.09, 5.18 ± 0.09 µg/mL respectively after 24, 48 and 72 hours) was found to be more active than ethanolic extract (CI_{50}: 15.59 ± 6.17; 9.61 ± 2.37; 6.26 ± 3.22 µg/mL respectively after 24, 48 and 72 hours) at all the incubation period. Interestingly, no significant difference was observed between the activities of hydroethanolic extract compared to that metronidazole (CI_{50}: 8.42 ± 0.44, 6.45 ± 0.22 and 3.42 ± 0.33 µg/mL respectively after 24, 48 and 72 hours) (the reference drug).

3.3. Antioxidant Activities of the Studied Plant Extracts

3.3.1. DPPH Free Radical Scavenging Activity

The antiradical activity was evaluated throughout the scavenging of the DPPH radical. *C. variegatum* leaf extracts and fractions effectively trap the DPPH radical in a concentration-dependent manner between 1 and 500 µg/mL (Figure 4(a)). The IC_{50} revealed that the extracts and fractions exhibited moderate antiradical potential but this remained lower compared to ascorbic acid. The fractionation process decreased the DPPH antiradical activity of hydroethanolic extract and increased that of ethanolic extract.

3.3.2. Lipid Peroxidation Inhibitory Activity

The ability of the different extracts and fractions of *Codiaeum variegatum* to inhibit membrane lipid peroxidation was effective (Figure 4(b)). Moreover, the ethanolic extract shows the highest inhibitory activity. However, ascorbic acid

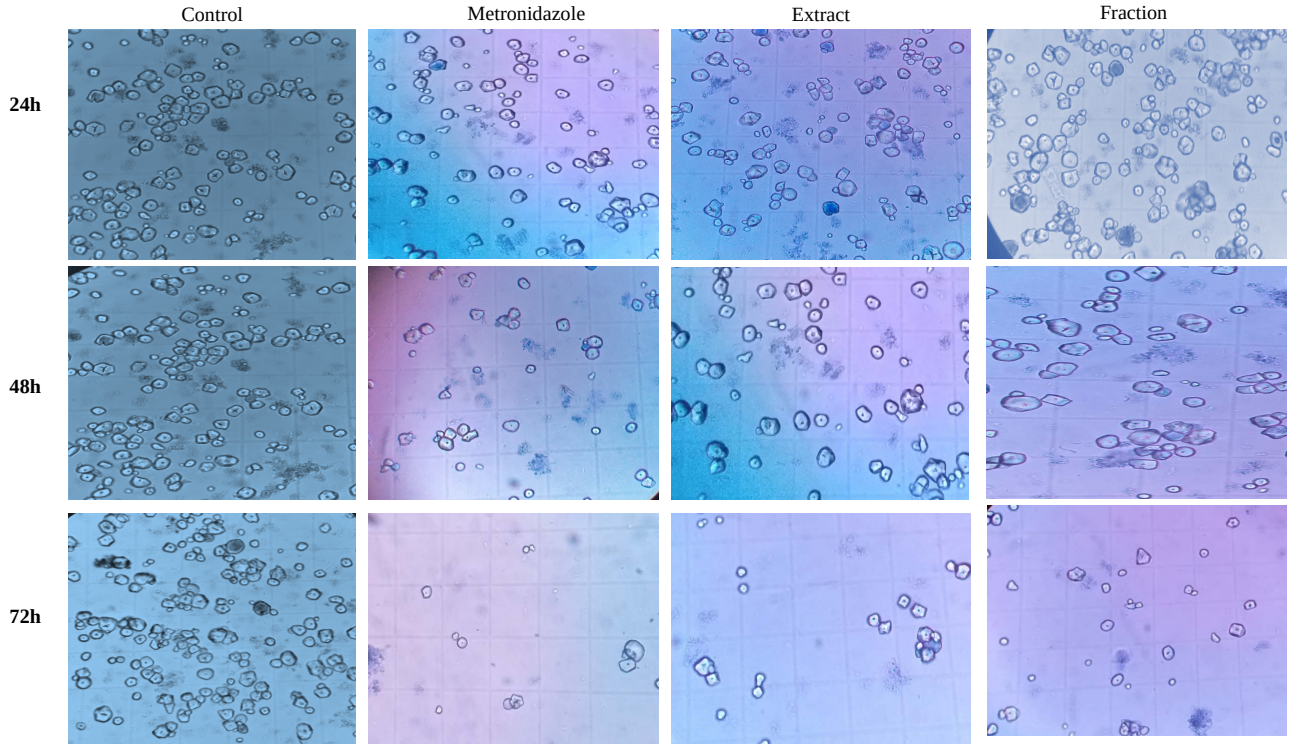

Figure 2. Variation of E. histolytica population number in the presence of *C. variegatum* extracts, fractions, and Metronidazole against control observed under 40× optical microscope after trypan blue staining.

Table 2. Anti-amoebic efficacy of *C. variegatum* extract and fractions through IC_{50} determination.

	IC_{50} in µg/ml (Mean ± SD)		
Extracts/Fractions	24 hours	48 hours	72 hours
HE	10.08 ± 0.42*	5.18 ± 0.09*	3.72 ± 0.11*
EE	17.59 ± 2.17	9.61 ± 2.37*	6.26 ± 1.22
HEF1	81.17 ± 9.72	45.69 ± 3.59	17.14 ± 0.23
HEF2	86.18 ± 5.84	43.22 ± 4.06	10.03 ± 0.85
HEF3	89.07 ± 4.40	41.34 ± 4.21	14.84 ± 1.04
HEF4	85.38 ± 11.05	41.25 ± 5.28	18.31 ± 3.69
HEF5	ND	74.27 ± 3.36	55.51 ± 6.42
EEF1	81.4 ± 19.5	50.1 ± 10.2	14.00 ± 4.01
EEF2	ND	89 ± 5.06	39.02 ± 2.14
EEF3	63.52 ± 14.06	44.85 ± 10.20	15.00 ± 1.25
EEF4	ND	ND	59.37 ± 0.37
EEF5	71.00 ± 8.42	46.95 ± 6.45	18.40 ± 2.16
MTZ	8.42 ± 0.44	6.45 ± 0.22	3.42 ± 0.33

* = value significantly non different from the standard drug MTZ.

activity was more pronounced compared to that extract (respectively 21.52 ± 9.87 µg/mL and 10.26 ± 4.02 µg/mL). Three fractions (EEF1, EEF3, and EEF5) exhibited moderate inhibitory potentials.

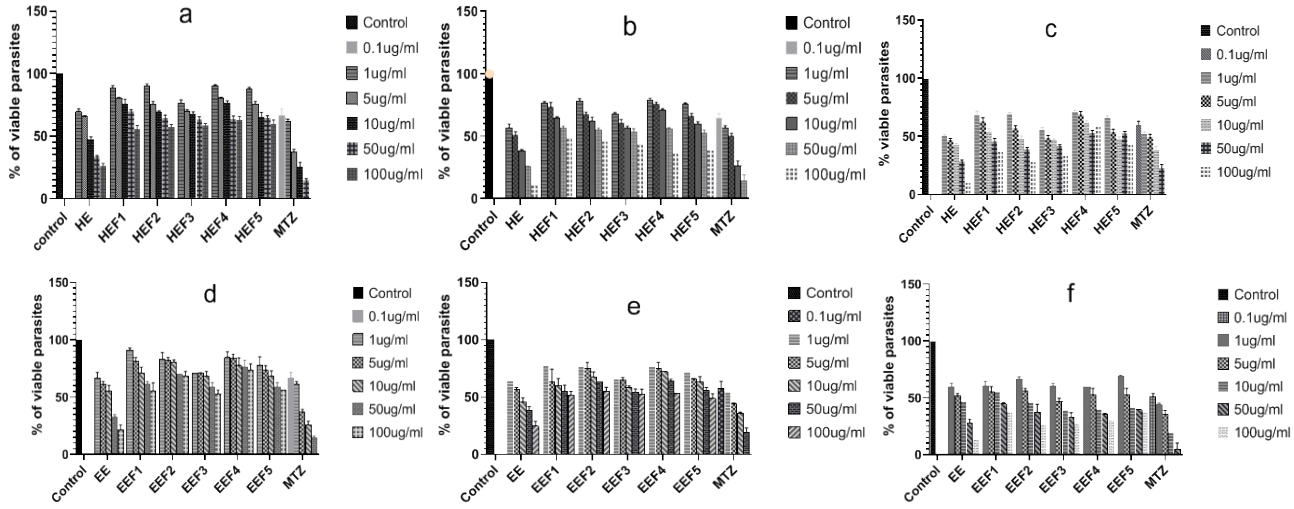

Figure 3. Effect of *C. variegatum* extracts, fractions and metronidazole against clinical isolate of *E. histolytica* growth on polyxenic culture after (a) 24 hours; (b) 48 hours and (c) 72 hours of incubation.

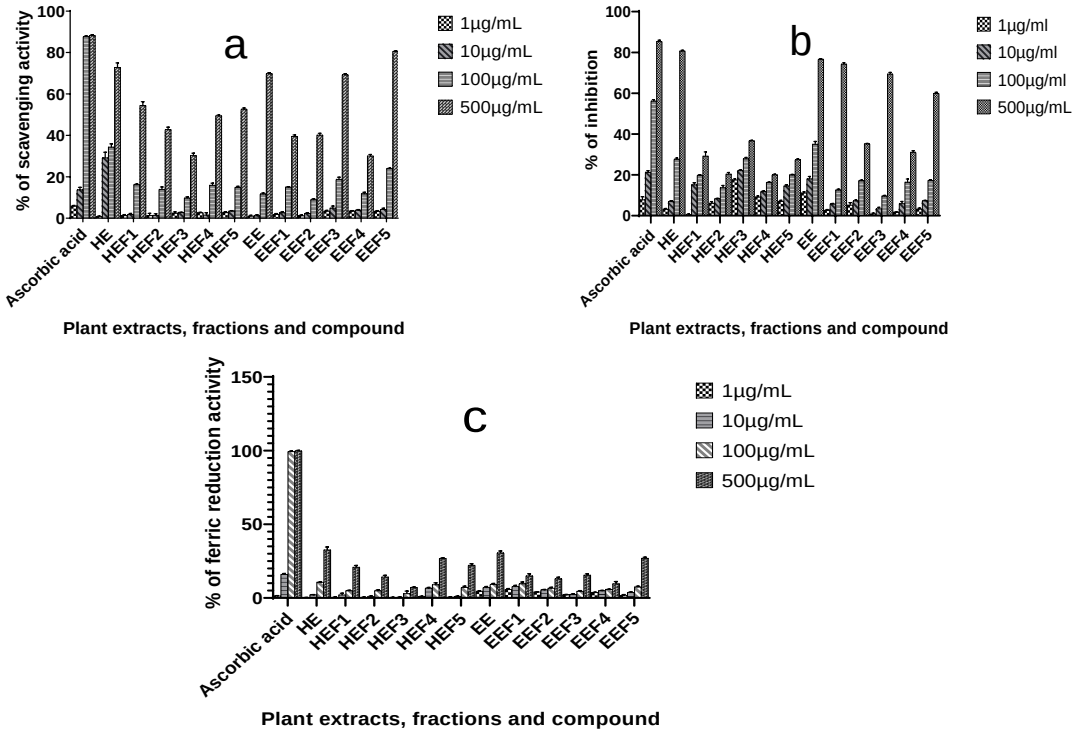

Figure 4. Antioxidant potential of *C. variegatum* extracts and fractions through (a) DPPH free radical scavenging activity; (b) inhibition of lipid peroxidation; (c) ferric reducing activity.

3.3.3. Ferric Reducing Activity (FRAP) of *Codiaeum variegatum* Stem Extracts

The ferric reducing power assay revealed that ethanolic extract exhibited the highest activity extract with an evolution going in a concentration-dependent manner (Figure 4(c)) However the activity of ethanolic extract was lower than that of ascorbic acid (543.60 ± 42.52 µg/mL and 21.34 ± 2.91 µg/mL respectively) (Table 3). All the fractions exhibited very low ferric reducing powers.

Table 3. Antioxidant efficacy of *C. variegatum* extracts and fractions through IC_{50} determination.

	IC_{50} ± SD (µg/ml)		
Extracts/Fractions	**DPPH scavenging activity**	**Lipid peroxidation inhibition (MDA)**	**Ferric reducing power (FRAP)**
HE	142.8 ± 14.02	189.40 ± 6.81	>500
EE	311.50 ± 4.12	144.30 ± 11.33	>500
HEF1	427.10 ± 6.37	>500	>500
HEF2	>500	>500	>500
HEF3	>500	>500	>500
HEF4	>500	>500	>500
HEF5	457.90 ± 8.83	>500	>500
EEF1	>500	282.00 ± 7.84	>500
EEF2	>500	>500	>500
EEF3	279.90 ± 7.60	324.00 ± 6.25	>500
EEF4	>500	>500	>500
EEF5	205.30 ± 6.05	359.00 ± 10.39	>500
Ascorbic Acid	31.20 ± 4.39	61.34 ± 4.42	17.68 ± 4.42

3.3.4. Total Antioxidant Capacity of *Codiaeum variegatum* Stem Extracts

The determination of the total antioxidant capacity was done by the phosphomolybdenum method, the results expressed in milligram ascorbic acid equivalent per gram of plant extract (mgEAA/g extract) showed us that hydroethanolic extract has the greatest ability to reduce the phosphomolybdic complex (54.77 ± 0.08 mgEq AA/g) followed by EEF5 (44.00 ± 0.01 mgEqAA/g) (Table 4). EH and EEF2 exhibited the lowest phosphomolybdic reducing power (13.72 ± 0.0 mgEq AA/g and 12.91 ± 0.13 mgEq AA/g respectively.

3.3.5. Total Phenolic Compound and Total Flavonoid Contents

Total phenolic and flavonoid contents were determined for the extracts and fractions of *C. variegatum*. It was noticed that the phenolic ranged from 2 mgGAE/g to 35 mgGAE/g and flavonoid contents ranged from 0.04 mgQE/g to 2 mgQE/g (Table 4). EEF5 exhibited the highest phenolic compound content (35.13 ± 0.01 mgGAE/g) followed by the ethanolic (14.00 ± 0.02 mgGAE/g) and hydroethanolic (4.18 ± 0.04 mgGAE/g). Ethanolic fractions and extract exhibited higher flavonoid contents compared to hydroethanolic extract and fractions. No significant difference was found between the flavonoid content of hydroethanolic extract and those of its fractions.

4. Discussion

The search for the efficacy of plant extracts and determination of their mechanism of action are major and permanent challenges for the valorization of phytotherapy

Table 4. Total antioxidant capacity, total phenolic compound content, and total flavonoid content of *C. variegatum* extracts and fractions.

Extracts/Fractions	TAC mgAAE/g (Mean ± SD)	TPC in mgGAE/g (Mean ± SD)	TFC in mgQE/g (Mean ± SD)
HE	54.77 ± 0.58	4.18 ± 0.04	2.09 ± 0.07
EE	13.72 ± 0.01	14.00 ± 0.02	0.35 ± 0.02
HEF1	32.51 ± 0.13	2.65 ± 0.63	2.04 ± 0.02
HEF2	39.77 ± 0.13	2.58 ± 0.05	1.69 ± 0.12
HEF3	32.85 ± 0.43	3.60 ± 0.04	1.73 ± 0.14
HEF4	29.80 ± 0.59	2.65 ± 0.06	1.36 ± 0.05
HEF5	30.51 ± 0.55	2.59 ± 0.05	2.13 ± 0.01
EEF1	39.00 ± 0.24*	3.84 ± 0.10	0.16 ± 0.01
EEF2	12.91 ± 0.13	2.76 ± 0.02	0.04 ± 0.01
EEF3	16.55 ± 0.04	35.13 ± 1.26	0.26 ± 0.01
EEF4	23.14 ± 0.17	3.74 ± 0.02	0.23 ± 0.01
EEF5	44.00 ± 0.01	3.24 ± 0.25	0.33 ± 0.02

[34]. Thus a fractionation of the hydroethanolic and ethanolic extracts of Codiaeum variegatum was carried out and the effect of each fraction was evaluated on a polyxenic culture of a clinical isolate of *E. histolytica*. Although the axenic culture of HM1:IMSS strain is mostly used for antiamoebic assays [17] [35], polyxenic culture of *E. histolytica* clinical isolate was previously used in an *in vitro* model for the evaluation of the amoebicidal activity of extracts and fraction [18] [26] [36]. Upon fractionation of the extracts, the antiamoebic activity decreased. This decrease might be due to the heterogeneous distribution of biologically active secondary metabolites in the different fractions. Previously published research with the fractionation of crude aqueous extract of the same plant showed an increase in activity with regard to the fractionation process using different solvent system [17]. Among extracts, the hydroethanolic extract exhibited the highest antiamoebic activity. However, no significant difference was observed between the antiamoebic activities of extracts compared to that of metronidazole. These results are similar to those obtained with the aqueous leaf extract of *C. variegatum* against clinical isolates of *E. histolytica* trophozoites on polyxenic culture [18]. However, these activities are higher compared to those obtained with the aqueous extract of the same plant against HM1:IMSS strain of *E. histolytica* trophozoites on axenic culture [17], the aqueous leaf extract and fractions of *E. hirta* against the same isolates [26]. Forty plant extracts and their one hundred and twenty fractions were studied for the antiamoebic activity against clinical isolates of E. histolytica, and the results obtained were similar to those of the present finding [37]. During *E. histolytica* invasion the contact with intestinal epithelial cells triggers the host innate immune response leading to the secretions of pro-inflammatory mediators and pro-oxidants such as reactive oxygen

species (ROS) and reactive nitrogen species (RNS) by macrophages [38] [39]. An unbalance between the secreted pro-oxidant and host antioxidant may lead to DNA, proteins and lipids damage as well as the alteration of their functions [40]. To explain other health benefit of *C. variegatum*, the antioxidant potentials of the plant were assayed through DPPH-scavenging radical, inhibition of lipid peroxidation, FRAP and total antioxidant capacity assays followed by quantitative analysis. Extracts and fractions exhibited weak antioxidant activity within the limits of the tests carried out as compared to those of Ascorbic acid. Although the scavenging ability of DPPH free radical is widely used to analyze the antioxidant potential of naturally derived foods and plants, DPPH is not specific to any particular class of antioxidants, and thus provides the overall antioxidant capacity of the sample [41]. In fact, the scavenging of DPPH free radical implies the activity of hydroxyl groups carried by secondary metabolites in plant extracts [42]. The use of biochemical assays to assess the antioxidant power of plants has emerged and become the best reliable and readily available methods. Because of variable response engendered by a specific antioxidant in various testing systems, it is important to utilize diverse antioxidant assays to appreciate the mechanism of action of the bioactive principle involved [43]. The FRAP, Malonedialdehyde and the phosphomolybdenum assays are good indicators to achieve such work [44]. Extracts were found to be more effective than fractions for all the above mentioned tests, ranging from moderate to low activities as compared to vitamin C. previous work reported higher antioxidant activity of the stem hydroethanolic extract of the same plant [21]. Phenolic compounds may constitute the main class of natural antioxidants present in plants, food and beverages [45]. These have been investigated mainly due to their ability to delay or inhibit the oxidation process and inflammatory disorders, as consequences of some cellular pathological conditions [46] [47]. In the present study, it was found that ethanolic extract and EEF5 fraction exhibited the higher phnolic compound contents than hydroethanolic extract and other fractions. The results are similar to those obtained previously with the aqueous extract of 31 plant species with the highest antioxidant activity [48] [49]. However, flavonoid contents in hydroethanolic extract and fractions were higher than those of ethanolic extract and fractions. The result confirmed the moderate antioxidant activity of *C. variegatum* hydroethanolic and ethanolic extracts and fractions [50].

5. Conclusion

The present result brings a new approach to the ethnopharmacological uses of the plant as antidiarrheal in cases associated with Amoebiasis in Western Cameroun. Further investigations regarding the mode of action and other related pharmacological studies such as *in vivo* investigation, drug formulation and clinical trials are highly recommended.

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Disclosure

The study was independently designed by the authors and the funding body had no role in Lab experiments, analysis and interpretation of the data.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Author Contributions

SNP, CMF, SK, NMC and EMN carried out all experiments reported in the manuscript. SNP, SK, FNN and PFM designed the study. All authors read and approved the final manuscript.

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Abbreviation

EE: Ethanolic extract of *C. variegatum*
HE: Hydroethanolic extract of *C. variegatum*,
EEF1: fractions of ethanolic extract;
EEF2: fractions of ethanolic extract;
EEF3: fractions of ethanolic extract;
EEF4: fractions of ethanolic extract;
EEF5: fractions of ethanolic extract;
HE: Hydroethanolic extract of *C. variegatum*;
HEF1: fraction hydroethanolic extract of *C. variegatum*;
HEF2: fraction hydroethanolic extract of *C. variegatum*
HEF3: fraction hydroethanolic extract of *C. variegatum*
HEF4: fraction hydroethanolic extract of *C. variegatum*
HEF5: fraction hydroethanolic extract of *C. variegatum*
MTZ: Metronidazole;
TAC: Total antioxidant capacity
TPC: Total phenolic compound content
TFC: Total flavonoid content
AAE: Ascorbic acid equivalent
GAE: Gallic acid equivalent
QE: Quercetin equivalent