*Original Research Article*

# Anti-inflammatory and antimicrobial potential of three natural polyketides isolated from Endophytic fungus *Phomopsis sp* CAM212 against to dysenteric causing pathogens


## ABSTRACT:

**Aims:** The present work aimed to evaluate the anti-amoebic, antibacterial, and anti-inflammatory potential of three natural polyketides from *Phomopsis sp*. CAM212.
**Study design:** Clinical isolates of *E.histolytica, E.coli* ATCC25922 strain, primary peritoneal mouse macrophages and three polyketides were used.
**Places and duration of study:** Laboratory of Pharmacology and Toxicology, Laboratory of Medical Microbiology, Faculty of Science, University of Yaounde 1 between May and December 2022.
**Methodology:** During this work, we evaluated the ability of three natural polyketides from *Phomopsis sp* to inhibit the growth of germs responsible for amoebic and bacillary dysentery. First, the anti-amoebic activity was carried out on clinical isolates of *E. histolytica* in polyxenic culture. Subsequently, we evaluated the antibacterial potential on a strain of *E. coli* ATCC25922. Finally, the anti-inflammatory potentials were evaluated on a primary culture of SC activated macrophages through inhibition of nitric oxide (NO) production, activation of phosphatase alcaline (ALP) and inhibition of 5-lipoxygenase (5-LOX).
**Results:** It emerges from this work that among compounds, phomopsinin B, presented the highest anti-amoebic potential (84.4 % inhibition after 72h) and the highest antibacterial potential (MIC=12.5µg/mL and MBC/MIC=2). Phomopsini A and phomopsini A acetate showed moderate anti amoebic and antibacterial potentials. However, all these activities remain lower than that of metronidazole and ciprofloxacin (90% of amoebic inhibition after 72h; MIC=0.72µg/mL and MBC/MIC=4). Subsequently, all tested compounds were nontoxic on primary macrophages. Phomopsinin B exhibited a great anti-inflammatory potential through the inhibition of NO production ($IC_{50}$=1.72±0.91µg/mL); inihibition of 5-LOX activity ($IC_{50}$=36.97±7.12µg/mL) and activation of ALP activity ($IC_{50}$=0.13±0.01µg/mL) as compared to Baicalin the standard. The anti-inflammatory potential of phomopsinin A and phomopsinin A acetate were lower compared to baicalin.
**Conclusion:** Ultimately, among compounds tested, phomopsinin B exhibited the best anti-amoebic, antibacterial and ant-inflammatory potential similar to the respective standards within the limits of the tests carried out.


**Keywords:** polyketides, anti-amoebic, antibacterial, anti-inflammatory, dysentery.


## 1. INTRODUCTION

Dysenteries are endemo-epidemic diseases of a microbial origin leading to an ulcerous inflammation of the large intestine and characterized by frequent evacuations of bloody mucus accompanied by violent colitis [1]. These pathologies are frequently linked to numerous enteric germs, including the bacterium *Escherichia coli* (E. coli) responsible for bacillary dysentery and the protozoan *Entamoeba histolytica* (*E. histolytica*) causing amoebic dysentery [2]. *E. coli* is a type of fecal coliform of the Enterobacteriaceae family divided into various pathotypes that cause different manifestations of the disease (traveler's diarrhea; infantile gastroenteritis, etc.). *E. histolytica* is a unicellular protozoan found in two forms during its evolutionary cycle: the mobile vegetative form and the resistant cystic form responsible to its dissemination. Epidemics caused by these germs result in significant morbidity and mortality, which are often underestimated. Indeed, nearly 4 million deaths are deplored in the world, including more than 525,000 children each year as a result of infectious dysentery [3]**.** Amoebiasis affects about 50 million people worldwide with a mortality rate approaching 100,000 deaths per year [4]**.** In the Republic of China,

*E. coli* bacillary dysentery was in the top 10 of 39 reported infectious diseases from 2004 to 2014 revealing a high frequency of bacillary dysentery in children under 5 years of age [5]. In Cameroon, according to recent studies conducted on parasitic infections; prevalences would be 33% in Yaounde, 27.8% in Douala, 59.5% in Dschang and 28.7% for HIV co-infected individuals [6, 7].

Pathogens responsible for dysentery are transmitted between humans via the fecal-oral route and create lesions in the intestinal wall that causes inflammation of the digestive tract regardless of the etiological agent. Intestinal inflammation is therefore 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 pathogenic agents [8]. Its essential role is the elimination of the pathogen and the repair of the injured tissue [9]. Moreover, inflammatory reactions are accompanied by the production of numerous enzymes such as alkaline phosphatase involved in the repression of the NF-κB signaling pathway of cellular inflammation and lipoxygenases that synthesize pro-inflammatory mediators such as leukotrienes from arachidonic acid [10]**.**

Furthermore, activated macrophages secrete various inflammatory mediators such as chemokines and cytokines (IL-1, IL-6, TNF-α, NO), all in an effort to eliminate antigen [11]. However, failure in antigen removal and repair of injured tissue causes overproduction of these mediators and in a persistent manner leading to progression into chronic inflammation that can be fatal to the organism [12]**.** Thus, the inhibition of these inflammatory mediators and enzymes appear to be an important target for the prevention of chronic inflammatory diseases. The conventional therapeutic armamentarium used for the treatment of dysentery mainly includes imidazole molecules for amebiasis and fluoroquinolone antibiotics for *E. coli* bacillary dysentery. These drugs may exhibit carcinogenic, teratogenic and mutagenic effects over time [13]. Also, this therapeutic treatment does not take care of the inflammatory response disturbances that are common during the physiopathological process of this diesease. Added to this are the economic costs associated with microbial resistance to *E. coli* frequently leading to therapeutic failures that result in complications and even death.

Due to the recurrence of toxicity and undesirable side effects of these synthetic molecules, it is important to direct the search for new therapeutic agents towards endophytic fungi which today constitute a potential resource of natural compounds [14]. In fact natural compounds are secondary metabolites from plants for the most part (80% of the secondary metabolite), bacteria, fungi and many marine organisms (sponges, tunicates, corals and snails). They produce various bioactive molecules grouped into several structural categories such as alkaloids, flavonoids, polyketides and terpenoids [15, 16]**.** Many compounds from endophytic fungi have already shown interesting pharmacological activities like those isolated from *Phomopsis* species.

Very few research works have investigated their anti-dysenteric potentials on pathogenic strains of *E. coli* and *E. histolytica*. Thus, in the continuous search for therapeutic alternatives based on natural antimicrobials and in the perspective of making available to local populations a wide range of biological, natural, effective and low cost products, we proposed to study the antimicrobial and anti-inflammatory potential of some natural polyketides from *Phomopsis* sp CAM212 on two types of dysenteries: amoebic and bacillary dysenteries.

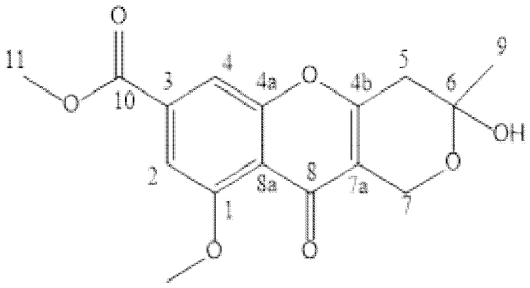
## 2. MATERIAL AND METHODS

### 2.1 Biological material

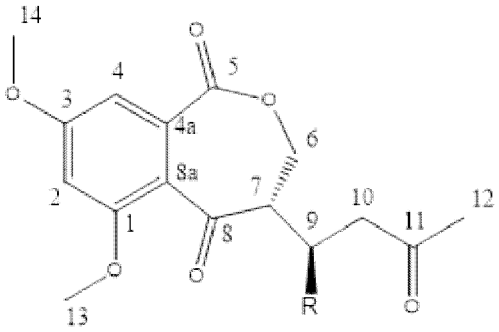
The biological animal material used consisted of macrophages prepared from mice, clinical isolates of *E. histolytica* maintained on polyxenic culture at the Laboratory of Pharmacology and Toxicology of the Faculty of Sciences, University of Yaounde 1 and the enteropathogenic strain of E. coli ATCC25922 maintained at the Laboratory for Phytobiochemistry and Medicinal Plant Study.

### 2.2 Preparation of polyketides natural compounds.

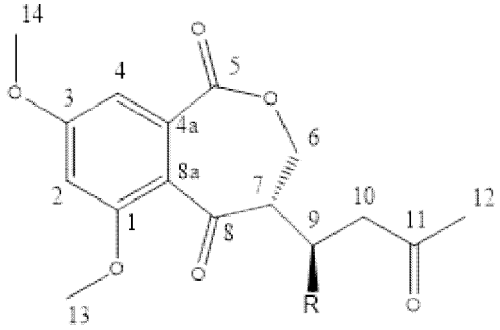
Polyketide natural compounds isolated from Phomopsis sp CAM 212 strains following the previously described protocole [17], were obtained from the Department of bioorganic chemistry, Leibniz Institute of Plant Biochemistry, Halle (Saale) Germany. The structures of tested compounds are shown in Figure1.




Phomopsinin B




R=OCOMe ; Phomopsinin A acetate




R=OH; Phomopsinin A

**Figure 1: Structures of natural polyketides isolated from the fungus *Phomopsis sp* CAM212**

## 2.3 Antiamoebic testing

### 2.3.1 Polyxenic culture of *Entamoeba histolytica*

A de Boeck and Drbohlav two-phase medium that involves a solid phase (Ringer's solution + egg) and a liquid phase (nutrient-containing lock solution) was used for polyxenic culture of *E. histolytica*. Prior to inoculation, complete media were pre-incubated at 37°C for 30 min and 10 µL of polyxenic culture maintained at the Laboratory of Pharmacology and Toxicology, University of Yaounde 1 containing clinical isolates of *E. histolytica* trophozoites were added to each tube. The tubes were incubated at 37°C and the growth of *E. histolytica* trophozoites was checked every 48 and 72 h. Then, the tubes were removed from the incubator and shaken to detach the parasites from the solid phase and left for 5 min, and the supernatant was decanted to remove the culture medium. The pellet containing the parasites was placed in a tube containing new pre-incubated medium and incubated as previously described [18]**.**

### 2.4.2 Assessment of amoebic viability

The Trypan blue counting method was used. For this purpose, tests were performed using clinical isolates of *E. histolytica* in polyxenic culture counted using the Malassez cell, harvested in log phase at a concentration of 1.67×107 cells/mL and inoculated into 2.5 mL of new culture medium in the presence of six compounds from *Phomopsis sp*. All compounds were tested at the concentration of 25 mg/mL during the course of the tests. Metronidazole used as the reference anti-amoebic compound was also tested at the concentration of 25 mg/mL. During these tests a control tube, a standard and test tubes were used. The control contained parasites incubated with sterile distilled water; the standard contained parasites incubated with metronidazole; and the test tubes contained parasites incubated with the different compounds. Before each incubation, each tube containing the new culture medium, previously introduced 30 min in the incubator, received a pinch of rice starch. The experiment was performed in triplicate for

each compound and all tubes were placed in the incubator at 37.5°C. Tubes removed from the incubator were immediately placed on ice. In a 1.5mL eppendorf tube; 25µL of parasite suspension was introduced and 225µL of 0.4% Trypan blue solution prepared in 0.9% NaCl was added. The whole mixture was homogenized by vortex. Then, 20µL of the mixture was introduced into the Malassez cell which was then covered with a glass slide [19]**.** Viable amoeba were counted under a light microscope at 40X magnification and the amoebic concentrations in the culture medium were calculated using the following formula: N= n × Nr × Vr × Fd

In which: N= concentration of viable amoebae (amoebae/mL); n= number of live amoebae counted in the Malassez cell; Fd= dilution factor; Nr = Number of rectangles (100); Vr = Volume of a rectangle (1000 mm3)

The percentages of inhibition were calculated using the following formula:

% d'inhibition = $\frac{Nt-NC}{NC}$ X 100 In which :

Nc = Number of living amoeba in the control tube; Nt = Number of living amoeba in the test tube.

After determining the percentages of inhibition, the percentages of viability were calculated according to the following formula: Percentage of viability = (100 - % inhibition)

## 2.4 Antibacterial testing

### 2.4.1 Formulation of Luria Bertani culture medium

One liter of liquid culture medium was prepared by adding 10 g of peptone, 5 g of yeast extract and 10 g of NaCl was added inside a volumetric flask containing 900 mL of sterile distilled water. The volume was completed to one liter using sterile distilled water then, followed by homogenization with a magnetic stirrer until the components of the medium were completely dissolved. The prepared medium was autoclaved for 15 min at 121°C for sterilization. For the preparation of LB agar culture medium, 15 g of Agar was added to the components of the liquid medium and prepared as previously described. The culture media were poured into petri dishes while hot; the dishes were then sealed with film paper. Finally; the media were stored in the refrigerator at +4°C until use [20]

### 2.4.2 Evaluation of bacterial activity

The determination of the inhibition parameters of the compounds was done according to the micro-dilution method on liquid media according to a previously described protocol M7-A7 [21]. 2-(4-iodophenylyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium (INT) was used for growth revelation of bacterial cells exposed to decreasing concentrations of compounds after incubation. A 96-well microplate, allows to study the activity in triplicate of two antimicrobial stock solutions on a bacterial strain. In each well of a sterile microplate placed horizontally, 100 µL of Luria-Bertani Broth (LBB) culture medium was introduced. Subsequently, 100 µL of an antimicrobial stock solution (compound or ciprofloxacin) was introduced into the first four wells (1A, 1B, 1C, 1D) of column 1. Thus there's three first test wells and one first negative control well. In lines 1 to 12, successive dilutions following a geometric progression of reason 2 were performed (from the wells of the first columns (1A, 1B, 1C and 1D) to the 11th well by taking after homogenization 100 µL of the previous well to put in the next well; thus varying the concentration range from 25 to 0.0244 µg/mL for the compounds and ciprofloxacin because they were tested at the same concentration (100 µg/mL). Finally, 100 µL of *E. coli* bacterial inoculum was added to each well except for those in lines D and E (used as negative controls) which were instead supplemented with 100 µL of LBB culture medium. The final volume of each well was 200 µL and the tests were performed in triplicate. The microplate wells containing only the antimicrobial and culture medium was used as a negative control and the wells in the 12th column containing only the culture medium and inoculum were used as positive controls for bacterial growth. The microplate was covered and sealed with film and incubated for 24 hours at 37.5°C. After incubation, microbial growth was demonstrated by adding 3 drops of 0.02% INT solution to two (02) of the three (03) test wells, the test wells of the non-INT labeled line were used for the determination of the MBC. MIC was defined as the lowest concentration of antimicrobial for which there was no bacterial growth visible to the naked eye (CLSI, 2015). For BMC determination, 50 µL of the unlabeled line test wells with a concentration greater than or equal to their MICs, were spiked into 150 µL of sterile Luria-Bertani broth contained in the microplate wells. The plate was incubated for 24 h at 37, 5°C. After incubation 3 drops of 0.02% INT were added to the wells and left for 15 min. The experiment was performed in duplicate. MBC was considered the lowest concentration of compound or ciprofloxacin for which no visible germ growth was observed. The MBC/MIC ratio was used to determine the

bacteriological profile of the tested compounds. As previoulys described when MBC/MIC < 4, the substance is considered to be bactericidal; and when 4 ≤ MBC/MIC ≤ 16 the substance is bacteriostatic [21]. MBC/MIC > 16: tolerant substance.

## 2.5 Determination of the in vitro anti-inflammatory potentials of natural polyketides on primary culture of peritoneal macrophages

### 2.5.1 Primary macrophages cells culturing

Macrophages were isolated and maintained in culture as described [22]**.** Mice were elicited by intraperitoneal injection of 0.5 mL of a 2% starch solution (inflammatory agent). Four days later, the animals were sacrificed by cervical dislocation. Then the primary peritoneal macrophages obtained by the previously described method were suspended in 2 mL of DMEM culture medium, and 25µL ($2.3\times10^7$ cells/mL) of the suspension were used for the Trypan blue viability assay. Counted cells were dispensed into 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 microplate was incubated for 1h at 37°C (5% $CO_2$), then 50 µL of compounds at different concentrations (0.1, 1, 10 and 100 µg/mL) were added to the test wells and 50 µL of DMEM was 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 nitric oxide assays while the pellets were used for alkaline phosphatase, 5-lipoxygenase and MTT cytotoxicity assays.

### 2.5.2 MTT Cell cytotoxicity

The cell pellet from the different incubations was taken up in 100 µL of MTT solution (0.5 mg/mL in PBS) and the mixture was incubated at 37°C for 1h 30 min, then the supernatant was removed and 100 µL of acidified isopropanol was added to each well to dissolve the formazan crystals formed. Finally, the absorbance of the blue-violet solution was read at 550 nm against the acidified isopropanol solution [23]**.** The percentages of cell viability were calculated using the following formula:

$$\%\ \text{of}\ Viability = \frac{\text{Sample OD}}{\text{Control OD}} \times 100$$

### 2.5.3 Evaluation of the effect on nitric oxide production

The supernatants obtained during the previous incubations were used for the realization of this test. Indeed, 100 µL of supernatant were mixed with 100 µL of Griess reagent (1% sulfanylamide, 0.1% naphthyl ethylene diamine dihydrochloride in 2.5% v/v phosphoric acid). The mixture was incubated (5% CO2) for 10 min and the absorbance was measured at 550 nm [24]**.** The amount of nitrite was measured against the calibration curve of the sodium nitrate standard.The percentage of inhibition of nitric oxide production was calculated according to the formula:

$$\%\ inhibition = \frac{(OD\ control - OD\ assay\ )}{OD\ control} \times 100$$

### 2.5.4 Evaluation of the effect on the alkaline phosphatase activity

The cell pellets obtained after the incubation of macrophages were used. The pellets obtained were solubilized by adding 25 µL of Triton X-100, followed by the addition of 50 µL of p-nitrophenylphosphate (10 mM) and 50 µL of glycine buffer (0.1 M, pH 9.0). All solutions were incubated (5% C02) for 30 min at 37°C. The reaction was stopped by adding 100 µL of NaOH buffer (0.2 M, pH 12) [25]**.** The absorbance was measured at 405 nm and the percentage change in lysosomal enzyme activity was calculated taking into account the control tubes according to the formula below:

$$\%\ \text{de variation de l'activité de l'enzyme lysosomale} : \frac{(DO\ assay - DO\ control}{DO\ control} \times 100$$

### 2.5.5 Evaluation of the effect on 5-lipoxygenase activity

The activity of 5-lipoxygenase was performed in sterile test tubes [10]. After isolating mouse macrophages and recovering in DMEM culture medium, 950µL of macrophage cells were introduced into each tube (100000 cells per tube). Then we added 300µL of Saccharomyces cerevisiae suspension (250µg/mL) to each tube, except for the negative control where the culture medium was added. This was followed by a first incubation of one hour at 37°C (5% CO2). Then 50µL of compounds at concentrations of 0, 1, 1, 10 and 100 µg/mL were introduced in the test tubes; 50µL of ascorbic acid, acetylsalicylic acid

and baicalin for the standard tubes and 50µL of DMEM culture medium for the control tubes; a second incubation of 3 hours at 37°C (5% $CO_2$) followed. After that we centrifuged each tube at 2000rpm for 10 minutes at 4°C and removed the supernatant. The pellet containing the cells was recovered in 50µL of Triton X-100 and then the tubes were vortexed within 2 minutes. Finally we added 1000µL of linoleic acid (125µM) and incubated for 30 minutes. All tests were performed in triplicate and the optical density of the supernatant was read at 234nm. The percentage of inhibition of the activity of this enzyme was calculated using the following formula:

% of inhibition: $\frac{(OD\ poitive\ control - OD\ assay)}{OD\ poitive\ control} \times 100$

## 2.6 Statistical analysis

Statistical analyses of the values obtained were performed using Graphpad Prism 9.0.0 software. The results were expressed as mean ± standard deviation and the different values were compared using the analysis of variances test "one-way ANOVA" with the Turkey multiple comparison test and the differences were considered significant for a p-value $p<0.05$.

# 3. RESULTS

## 3.1 Anti-amoebic potential

Clinical isolates of *E. histolytica* maintained on de Boeck and Drbohlav diphasic medium were incubated with different polyketides. The variation of trophozoite number with concentration and incubation time observed by light microscopy showed a significant decrease in pencentage of viable parasites in the tested tubes compared to the control tubes after 24h, 48 and 72h post-treatment(Table 1). Among compounds, phomopsinin B, presented the highest anti-amoebic potential (84.4 % of amoebic inhibition after 72h). Phomopsini A and phomopsini A acetate showed moderate anti amoebic potentials (respectively 64 and 73.86% of amoebic inhibition after 72h). However, all these activities remained lower than that of metronidazole (90% of amoebic inhibition after 72h).

Table 1: antiamoebic effect of natural polyketides against clinical isolates of *E. histolytica*.

| | Amoebicidal activities (% of viable parasites) | | |
|---|---|---|---|
| **Compounds** | **24 hours** | **48 hours** | **72 hours** |
| **Control** | 100 ± 0,00 | 100 ± 0,00 | 100 ± 0,00 |
| **phomopsinin A** | 40,04 ± 2,29 | 38,38 ± 1,02 | 35,24 ± 0,78 |
| **Phomopsinin A acétate** | 29,74 ± 0,82 | 29,19 ± 1,65 | 26,14 ± 0,86 |
| **Phomopsinin B** | 37,78 ± 1,50 | 24,94 ± 12,77 | 15,54 ± 3,66* |
| **Metronidazole (standard)** | 17,08 ± 5,18 | 13,15 ± 6,42 | 10,19 ± 3,85 |

*= Value significantly non different from the standard

## 3.2 Anti-bacterial potential

Determination of inhibition parameters of polyketide natural compounds was done by liquid microdilution technique described by CLSI (2015). Following this logic, the inhibition parameters (MIC, BMC) of compounds from Phomopsis species on the enteropathogenic *E. coli* strain ATCC25922 represented in (Table 2) were determined. Ciprofloxacin which was the refreference drug taken as standard showed the best antibacterial potential against enteropathogenic *E. coli* strain (MIC=0.72µg/mL and MBC/MIC=4). The highest antibacterial potential was observed with phomopsinin B (MIC=12.5µg/mL and MBC/MIC=2) among natural tested compounds. No significant difference was observed between the antibacterial potential of phomopsinin A and that of phompsinin A acetate (MIC=25µg/mL and MBC/MIC=1).

Table 2: Antibacterial potential of natural polyketides against *E. coli* strain ATCC25922.

| | Bactericidal activities | | |
|---|---|---|---|
| **Compounds** | **MIC (µg/mL)** | **MBC (µg/mL)** | **MBC/MIC** |
| **phomopsinin A** | 25 | 25 | 1 |
| **Phomopsinin A acétate** | 25 | 25 | 1 |
| **Phomopsinin B** | 12.5 | 25 | 2 |

| | | | |
|---|---|---|---|
| **Ciprofloxacin (standard)** | 0.718 | 3.12 | 4 |

MIC= minimal inhibitory concentration; MBC= minimal bactericidal concentration

### 3.3 Anti-inflammatory activity

#### 3.3.1 Effect on macrophage viability

Evaluation of the cytotoxicity of natural compounds from Phomopsis sp and plants on primary macrophages was done in MTT at different concentrations of the compounds (Figure 2). The results revealed that the viability of macrophages in the presence of the different compounds showed no significant difference except in the presence of phomopsinin B compounds at the concentration of 100 µg/mL although the percentage of viability did not go below 80%. Then the concentration of 100 µg/mL was choosen as the highest testing concetration for the anti-inflammatory assays.

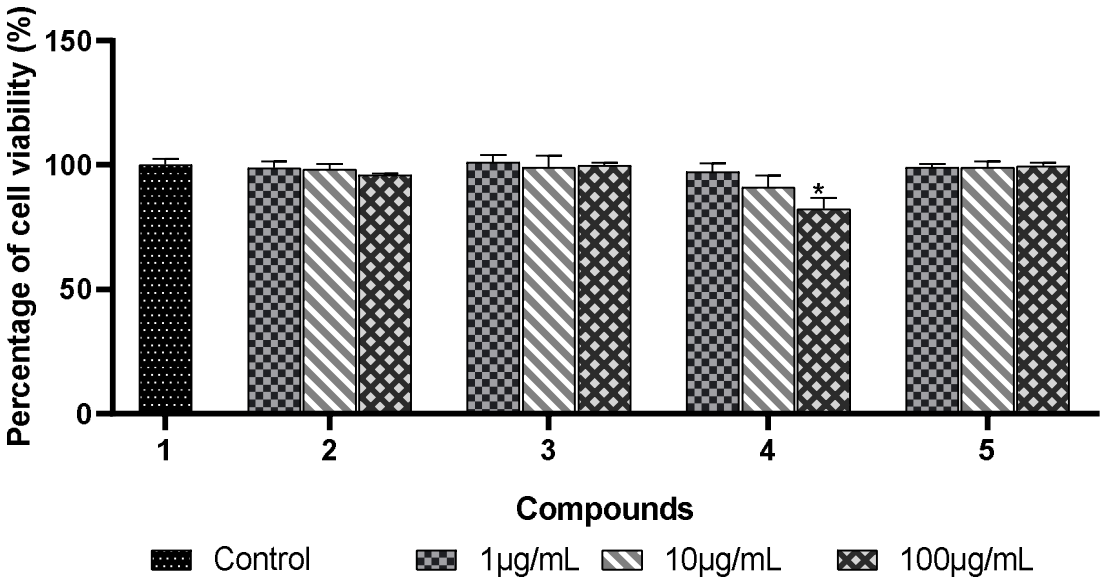



**Figure 2:** Effect of polyketides on the viability of primary mouse macrophages
1= Control; 2= Phomopsinin A; 3= Phomopsinin A acetate; 4= Phomopsinin B 5= Baicalin
*= value significantly different from the control ($p<0.05$).

#### 3.3.2 Inhibitory effect on nitric oxide production

Natural polyketides from Phomopsis sp effectively inhibit nitrite oxide production and the inhibitory effect was concentration dependent (Figure 3). Determination of IC50(Table 3) demonstrated that phomopsinin B showed the best inhibitory potential on the synthesis of this mediator ($IC_{50}$=1.72±0.91µg/mL). Phomopsinin A (8.05±2.19µg/mL) exhibited a moderate inhibitory potential and phomopsinin A acetate showed the lowest inhibitory potential (41.61±7.28 µg/mL). the inhibitory potential of phomopsinin B was significantly higher as compared to that of baicalin (4.26±0.93 µg/mL; $p<0.05$) the reference drug taken as standard.

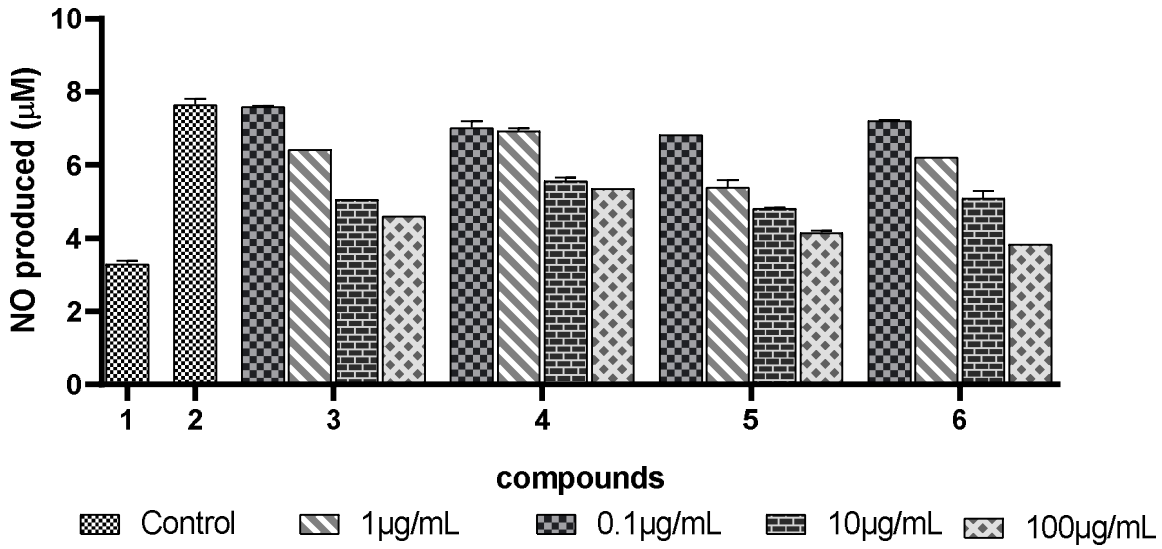



**Figure 3:** Inhibitory effect of natural polyketides on NO production.
1= Cells; 2= Cells + Saccharomyces cerevisiae 3= Phomopsinin A; 4= Phomopsinin A acetate; 5= Phomopsinin B 6= Baicalin

### 3.3.3 Stimulating effect on alkalin phosphatase (alp) activity

Phomopsis sp polyketides effectively boost the activity of this lysosomal enzyme (Figure 4) in a concentration-dependent manner. The determination of $IC_{50}$ (Table 3) showed that no significant difference was observed between the modulatory effect of Phomopsinin A ($IC_{50}$=0.63±0.08µg/mL), phomopsinin B ($IC_{50}$=0.13±0.01µg/mL) and baicalin ($IC_{50}$=0.92±0.09µg/mL) ($p<0.05$). hower phomopsinin A acetate ($IC_{50}$=2.03±0.28µg/mL) exhibited a good modulatory actyivity on ALP, this remained lower compared to baicalin.

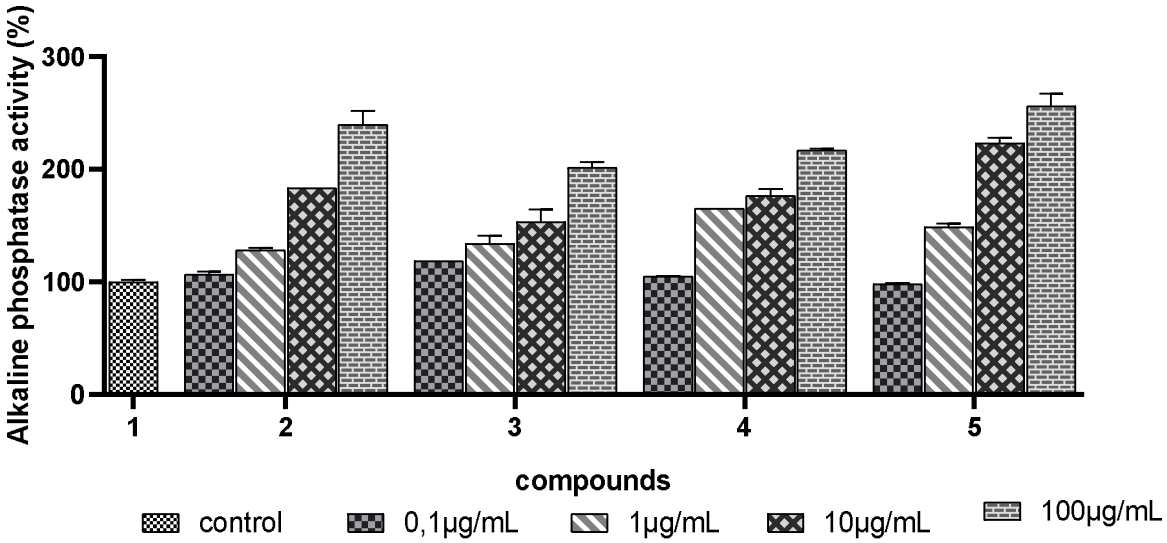



**Figure 4**: Effect of natural polyketides on the alkalin phosphatase activity
1 = Cells + Saccharomyces cerevisiae; 2 = Phomopsinin A; 3 = Phomopsinin A acetate; 4= Phomopsinin B 5 = Baicalin

### 3.3.4 Inhibitory effect on 5-lipoxigenase activity

Compounds from Phomopsis sp effectively inhibit 5-lipoxygenase activity and the inhibitory effect was concentration dependent (Figure 5). Determination of $IC_{50}$ (Table 3) showed that the phomopsinin A ($IC_{50}$=47.47±6.45µg/mL) phomopsinin B ($IC_{50}$=36.97±3.12µg/mL) presented the moderate inhibitory potentials on the activity of this enzyme. Those inhibitory potentials were significantly lower compared to that of baicalin ($IC_{50}$=10.77±2.16µg/mL) ($p<0.05$). no inhibitory effect was observed with Phomopsini A acetate.

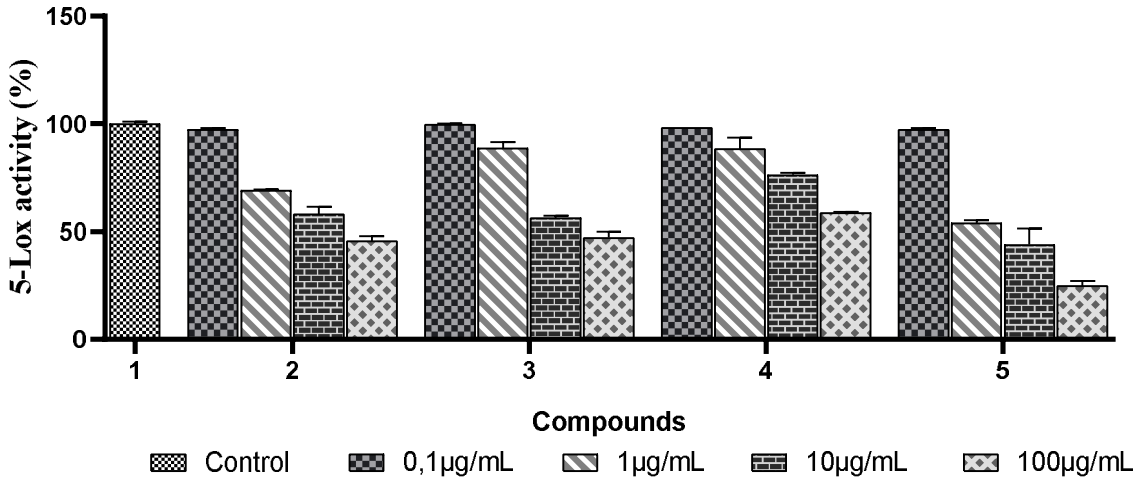



Figure 5: Inhibitory effect of natural polyketides on 5-lipoxigenase activity.
1 = Cells + Saccharomyces cerevisiae; 2 = Phomopsinin A; 3 = Phomopsinin A acetate; 4= Phomopsinin B 5 = Baicalin.

Table 3: efficacy of natural polyketides on the modulation of pro-inflammatory mediators

| | IC50 (µg/mL) | | |
|---|---|---|---|
| **Compounds** | **Inhibition of NO** | **Inhibition of 5-LOX** | **Activation of ALP** |
| **Phomopsinin A** | 8,05±2,19** | 47,47±6,45 | 0,63±0,08** |
| **Phomopsinin A acétate** | 41,61±7,28 | >100 | 2,03±0,28 |
| **Phomopsinin B** | 1,72±0,91* | 36,97±3,12 | 0,13±0,01** |
| **Baicalin** | 4,26±0,93 | 10,77±2,16 | 0,92±0,09 |

*= value significanly lower than the standard; **=Value significantly non different from the standard

## 4. DISCUSSION

Endophytic fungi are becoming more and more interesting to explore in the discovery of new therapeutic alternatives due to their great capacities to produce this wide range of biologically active compounds [26]**.** Indeed, following the recurrence of toxicity and undesirable side effects of synthetic molecules used in the treatment of dysenteric diseases, the effect of three natural polyketides isolated from Phomopsis sp CAM212 was evaluated in this work on a polyxenic culture of *E. histolytica*. The polyxenic culture medium is a commonly used in vitro model for culturing clinical isolates of *E. histolytica* and performing anti-amoebic tests [27, 28]. Indeed, the parasites grow in this environment in the presence of bacteria and yeasts that they use as protein sources; rice starch being the main energy source. During this study it was found an effectiveness of the anti-amoebic activity of natural polyketides through the inhibition of the

of *E. histolytica* growth. From the results obtained, it appears that phomopsinin B presented the highest anti-amoebic potential (84.4% inhibition after 72h) not significantly different from that of metronidazole (90% inhibition after 72h. These results are similar to those previously obtained [8] which demonstrated an inhibitory activity of *Sida rhombifolia* extracts on *E. histolytica* polyxenic culture. In addition, many studies have demonstrated that endophytic fungi are the origin of several antiparasitic molecules. One example is the endophytic fungus *Aspergillus terreus*-F7, associated with *Hyptis suaveolens* (L.) against *Schistosoma mansoni, Leishmania amazonensis* and *Trypanosoma cruzi*. The results showed that the three molecules, terrein, butyrolactone I and V from this endophyte killed at concentrations of 1297.3, 235.6 and 454.1 µM, after 24, 48 and 72 hours, 100% of *Schistosoma mansoni* worms. They also had moderate leishmanicidal activity with IC50s ranging from 78.6, 26.0 and 23.7 to µM respectively. Against *Trypanosoma cruzi*, only butyrolactone I and V were active and killed 100% of the cells at concentrations of 94.2 and 181.6 µM respectively [29]. Finally, it could be observed an increase in activity in some compounds with the acetate moiety. This is the case of phomopsinin A acetate having a percentage of parasite inhibition of 73.86% after 72h of incubation compared to phomopsinin A without the acetate moiety and having a percentage of parasitic inhibition of 64, 76%. The increase in efficacy observed during the tests suggests that the compounds' constituents may act synergistically on single or multiple targets associated with the physiological process leading to the destruction of *E. histolytica* trophozoites. Previously published work demonstrated that the use of phomopsinin A acetate obtained after acetylation of phomopsinin A significantly increased the activity of the acetylated compound in the nitric oxide assay in LPS-stimulated RAW 264.7 macrophages [30]. *E. coli* bacillary dysentery due to its various pathotypes remains a global public health concern. In the present work, Luria-Bertani culture medium is a nutrient culture medium used in many work for bacterial culture of pathogenic *E. coli* and recently in an interaction study between entoropathogenic *E. coli* (*E. coli* ATCC25922 a virulent enteropathogenic strain usually used in antibacterial tests) and *E. histolytica* [20; 31]. The results obtained revealed that natural polyketides isolated from phomopsis sp CAM212 exhibited bactericidal activity against *E. coli* ATCC25922 strainnwith MICs ranging from 12.5 µg/mL to 25 µg/mL compared to ciprofloxacin (MIC= 0.7812 µg/mL). The observed low sensitivity to E. coli strain could be attributed to the different constituents of the bacterial cell wall [32]**.** Indeed, the cell wall of Gram-negative bacteria is complex. It consists of a periplasmic space and a thin layer of peptidoglycan adjacent to the cytoplasmic membrane, it is also surrounded by an additional outer membrane composed of phospholipids and lipopolysaccharides [33]**,** which could make the cell wall impermeable to bioactive compounds. This would therefore tend to expel compounds from cells by acting as a selective barrier [34]**.** Two isocoumarins isolated from Xylaria species exhibited also antibacterial potential on the same *E. coli* strain with MICs ranging from 12.5 to 25 µg/mL [35]. Another study on the same *E. coli* strain ATCC25922 showed inhibitory activity with an MIC of 100 µg/mL using three alkaloids from *Fusarium proliferatum* [36]. On the other hand, low antibacterial potentials were observed when studying antibacterial activities on several Vibrio cholerae and shigella strains using compounds from *Phomopsis* CAM240 that revealed MICs ranging from 512 to 218 µg/mL [37]**.**

Macrophages play an important role in the body's defense system by triggering the inflammatory response through the release of several pro- and anti-inflammatory mediators (NO, TNFα, Il-1, Il-6, Il-10, TGFβ). These can act through the production or inhibition of reactive oxygen species and the activation of enzymatic pathways (Lipoxygenases, cyclooxygenases) [38] in order to eliminate a pathogen. Excessive release of these mediators is a risk factor for chronic inflammation, implicated in the pathogenesis of many human diseases [39]**.** Therefore, in this work the effect of natural polyketides on the production of some pro-inflammatory mediators was also evaluated. Cytotoxicity was performed using MTT which is a tetrazolium salt that mainly targets the effectiveness of the respiratory process through the activity of mitochondrial succinate dehydrogenase. It is only from the concentration of 100µg/mL that we started to observe an effective cytotoxicity especially for Phomopsinin B. This therefore showed a slight decrease in mitochondrial succinate dehydrogenase activity from this concentration. This concentration was therefore set as the maximum working concentration for the following. It was found a significant ($p<0.05$) decrease in the amount of NO produced by our macrophages compared with the control and in a concentration-dependent manner. Nitric oxide being a pro-inflammatory mediator synthesized by NO synthase from arginine and involved in vasodilation of blood vessels during the inflammatory response [40]. Inhibition of its synthesis is therefore a sign of anti-inflammatory activity; thus, phomopsinin B showed the best inhibitory activity (IC50= 1.72±0.91µg compound/mL). These results are similar to those of [17] who had observed a significant decrease in the amount of NO produced by LPS-stimulated RAW 264.7

macrophages in the presence of natural polyketides from Phomopsis species. Subsequently, the immunomodulatory activity of these compounds was evaluated through their effect on the activity of alkaline phosphatase. It appeared that these natural polyketides have the ability to boost the activity of this lysosomal enzyme compared to normal with phomopsinin B which boosted more effectively this enzyme (IC50=0.14±0.15µg/mL). Alkaline phosphatase is involved in the processes of repression of the nuclear factor NFκB of cellular inflammation and in the dephosphorylation of bacterial compounds, so its activation would reflect the ability of the compounds to contribute to the protection of the intestinal barrier by modulating the composition of the microbiota through action on the microorganisms. Hydroethanolic extract of *Codiaeum variegatum* exhibited similar increase on the alkaline phosphatase activity (IC50=0.274 µg/mL). 5-lipoxygenase catalyzes the synthesis of leukotriene B4 (LTB4) responsible for the recruitment of immune cells at the site of inflammation. It was observed that phomopsinin B inhibited 5-lipoxygenase activity in a concentration-dependent manner (IC50=36.97±7.12µg/mL) and with a higher inhibitory activity than baicalin (IC50=4.77±2.16µg/mL). The inhibition of this enzyme by natural polyketides may reflect their abilities to prevent the influx of immune cells to the site of inflammation. These results are similar to those previously obtained with *Saba senegalensis* extracts [10] that exhibited a significant decrease in 5-lipoxygenase activity. In the other hand the ethanolic extract of *Sida rhombifolia* (L) presented low effect compared to polyketides in the decreasing activity of 5-lipoxygenase (IC50=73.22µg/mL) produced by Wistar mice macrophages [8].

## 5. CONCLUSION

These findings demonstrated that polyketides natural compounds from *Phomopsis sp* CAM 212 are potent anti-dysenteric and anti-inflammatory agents. However, further in silico and in vivo studies are needed to better elucidate the mode of action of those natural compounds.


## ACKNOWLEDGEMENTS

The Cameroonian Ministry of Higher Education for financial support throughout the special allowance for the modernization of research. PNS, is grateful to The World Academy of Science -TWAS- for mobility grants.


## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

## AUTHOR CONTRIBUTIONS

PNS, MMLG, EEB, EMN, JBJ and AAJP, carried all the experiments reported in the manuscript. PNS, AAJP, MNE, FNN, and MFP designed the study. All authors read and approved the final manuscript.

## Ethical Approval

The present study was approved by the Joint Institutional Review Board for Animal and Human Bioethics (Ethical Clearance No BTC-JIRB2022056). All procedures followed the Cameroon National Veterinary Laboratory guidelines.

## REFERENCES


1. Rajaona H (2003). Dysentery. Medical hepato-gastroenterology. 2nd cycle gastroenterology course, 3rd ed. Antananarivo. 2003: 35.

2. Boi K (2022). Antibiotic resistance profile of Escherichia coli and Salmonella spp strains isolated from enteric infections in children aged 0 to 15 years in the community: case of the CSCom of Yirimadjo, Bamako. University of Sciences, Techniques and Technologies of Bamako. Pharmacological faculty. Doctoral thesis.

3. Aubry P, Gaüzère B (2019). Infectious diarrhea, News 2019. Center René Labusquière. Institute of Tropical Medicine, University of Bordeaux. 33076 Bordeaux (France). 8 p.

4. Houpt E, Hung C, Petri W (2016). Entamoeba histolytica (Amebiasis). Infectious Diseases and Antimicrobial Agents. 3(24):24-28.

5. Chang Z, Zhang J, Ran L, Sun J, Liu F, Luo L, Zeng L, Wang L, Li Z, Yu H, Liao Q (2016). The changing epidemiology of bacillary dysentery and characteristics of antimicrobial resistance of Shigella isolated in China from 2004–2014. BioMedCentral Infectious Diseases.16:685.

6. Nkenfou C, Nana C, Payne V (2013). Intestinal parasitic infections in HIV infected and non-infected patients in a low HIV prevalence region, West-Cameroon. PLoS One. 8(2):e57914.

7. Pechangou NS, Upninder K, Kapil G, Rakesh S, Moundipa FP (2015). Molecular differentiation of Entamoeba Spp. isolated from Cameroonian human immunodeficiency virus (HIV) infected and uninfected patient. Academy Journal of Parasitology and Vector Biology.7(7): 139-150

8. Pechangou NS, Ndiandoukoue NR, Mfotie NE, Enang II EB, Njayou FN, Moundipa FP (2023). Comparative study of potent anti-amoebic and ant-inflammatory activities of different extracts from Sida rhombifolia (L). Investigational Medicinal Chemistry and Pharmacology.6(1): 70.

9. Ashley NT, Weil ZM, Nelson RJ (2012). Inflammation: mechanisms, costs a natural variation. Annual Review of Ecology, Evolution and systematics. 43: 385-406.

10. Yougbaré-Ziebrou MN, Ouédraogo N, Lompo M, Bationo H, Yaro B, Gnoula C, Sawadogo WR, Guissou IP (2015). Anti-inflammatory, analgesic and antioxidant activities of the aqueous extract of the leafy stems of Saba senegalensis Pichon (Apocynaceae). Phytotherapy. 8p.

11. Noack M, Kolopp-Sarda MN (2018). Cytokines and inflammation: physiology, physiopathology and therapeutic use. French Journal of Laboratories. 489(3): 28-37.

12. Lee HN, Surh YJ (2012). Therapeutic potential of resolvens in the prevention and treatment of inflammatory disorders. Biochemistry pharmacology. 84: 1340-1350.

13. Mfotie NE, Weber C, Hernandez-Cuevas NA, Hon CC, Janin Y, Kamini FGM, Paul F. Moundipa PF, Guillen N (2014). Bioassay-Guided fractionation of Extracts from Codiaeum variegatum against Entamoeba histolytica discovers compounds that modify expression of Ceramide biosynthesis related genes. PLOS Neglected Tropical Diseases. 8(1): e2607.

14. Sadrati N (2021). Isolation, identification and culture of endophytic fungi isolated from Algerian medicinal plants for the production of biologically active secondary metabolites. Doctoral thesis in Microbiology. Ferhat Abbas Sétif University 1 Faculty of Nature and Life Sciences.

15. Zheng YK, Qiao XG, Miao CP, Liu K, Chen YW, Xu LH, Zhao LX (2016). Diversity, distribution and biotechnological potential of endophytic fungi. Annual Microbiology. 66: 529–5

16. Rana KL, Kour D, Kaur T, Devi R, Negi C, Yadav AN, Yadav N, Singh K, Saxena AK (2020). Endophytic fungi from medicinal plants: biodiversity and biotechnological applications. In: Microbial Endophytes, Functional Biology and Applications. Elsevier Masson. pp:273-305.

17. Jouda JB, Mfotie NE, Mbazoa CD, Yuan ZZ, Meli LA, Wandjia J, Shionoe Y, Wangb F (2018). Lambertellin from Pycnoporus sanguineus MUCL 51321 and its anti-inflammatory effect via modulation of MAPK and NF-κB signaling pathways. Bioorganic chemistry. 80: 216-222.

18. Parija S, Rao R (1995). Stool culture as a diagnostic aid in the detection of Entamoeba histolytica in the faecal specimens. Indian Journal Pathology Microbiology. 38: 359-363.

19. Bansal S (1987). Carbohydrate metabolism in the rat peritoneal macrophages. Journal of Biological Sciences. 12: 415-420.

20. Luz A, Fernandez-Lopez, Gil-Becerril K, Galindo-Gomez S, Teresa-Garcia, Ximenez C, Leon-Coria A, Moreau F, Chadee K, Tsutsumi V (2019). Entamoeba histolytica interaction with enteropathogenic Escherichia coli increases parasite virulence and inflammation in amebiasis. Infectiology immunology. 87(00): 279-19.

21. Rakholiya KD, Kaneria MJ, Chanda SV (2015). In vitro assessment of novel antimicrobial from methanol extracts of matured seed karnel and leaf of Mangifera indica L. (Kesar Mango) for inhibition of Pseudomonas spp. and their synergistic potential. Amtrong Journal Drug Discovery. 5: 13-23.

22. Titus RG, Gueiros-Filho FJ, De Frietas LR, Berveley SM (1995). Development of a safe live Leishmania vaccine line by gene replacement. Proceding of the National Academy of Sciences. 92 :10267-10271

23. Cedillo-Rivera R, Ramirez A, Muñoz O (1992). A rapid colorimetric assay with the tetrazolium salt MTT and phenazine methosulfate (PMS) for viability of Entamoeba histolytica. Archives Medical Research. 23: 59-61

24. Sun J, Zhang X, Broderick M, Fein H (2003). Measurement of nitric oxide production in biological systems by using Griess reaction assay. 3(1): 276-284.

25. Suzuki Y, Orellana MA, Schreiber RD, Remington JS (1988). Interferon-gamma: the major médiator of resistance against toxoplasma gondii. Science. 240 (4851) :516-524.

549 26. Atiphasaworn P, Monggoot S, Gentekaki E, Brooks S, Pripdeevech P (2017). Antibacterial and
550 anti-oxidant constituents of extracts of endophytic fungi isolated from Ocimum basilicum var. thyrsiflora
551 leaves. Current Microbiology. 74 : 1185-93.

552 27. Moundipa F, Kamini G, Bilong BC, Bruchhaus I (2005). In vitro amoebicidal activity of some
553 medicinal plants of the Bamun region (Cameroon). African journal of traditionnal complementary and
554 alternative medicines. 2(2): 113-121.

555 28. Pechangou NS, Moundipa P, Sehgal R (2014). In vitro susceptibilities of the clinical isolate of
556 Entamoeba histolytica to Euphorbia hirta (Euphorbiaceae) aqueous extract and fractions. African Journal
557 of Microbiology Research. 8(36), pp. 3354-3361.

558 29. Da Silva IP, Brissow E, Kellner Filho LC, Senabio J, de Siqueira KA, Vandresen Filho S, Damasceno
559 JL, Mendes SA, Tavares DC, Magalhães LG (2017). Bioactive compounds of Aspergillus terreus-F7, an
560 endophytic fungus from Hyptis suaveolens (L.) Poit. World Journal Microbiology Biotechnology. 33: 62.

561 30. Jouda JB, Mfotie NE, Tanemossu FSA, Yuan ZZ, Qiang Z, Mbazoa CD, Wolfgang B, Guo-lin Z,
562 Wandji J, Fei W (2020). Natural polyketides isolated from the endophytic fungus Phomopsis sp. CAM 212
563 with a semisynthetic derivative downregulating the ERK/IκBα signaling pathways. Planta Medicine.

564 31. Minogue TD, Daligault HA, Davenport KW, Bishop-Lilly KA, Broomall SM, Bruce DC, Chain PS,
565 Chertkov O, Coyne SR, Freitas T, Frey KG, Gibbons HS, Jaissle J, Redden CL, Rosenzweig CN, Xu Y, Johnson
566 SL (2014). Complete genome assembly of Escherichia coli ATCC 25922, a serotype O6 reference strain.
567 Genome Announcements. 2(5): e00969-14.

568 32. Stefanovic O, Radojevic I, Vasic S, Comic LJ (2012). Antibacterial activity of naturally occurring
569 compounds from selected plants. In: Antimicrobial Agents. Bobbarala V, (eds). 1st edition. Rijeka:
570 InTech. pp: 1-24.

571 33. Praptiwi, Fathoni A, Ilyas M (2020). Diversity of endophytic fungi from Vernonia amygdalina,
572 their phenolic and flavonoid contents and bioactivities. Biodiversitas. 21: 436–441.

573 34. Sebola TE, Uche-Okereafor NC, Tapfuma KI, Mekuto L, Green E, Mavumengwana V (2019).
574 Evaluating antibacterial and anticancer activity of crude extracts of bacterial endophytes from Crinum
575 macowanii Baker bulbs. Microbiology open. 8:1–10.

576 35. Zheng N, Yao F, Liang X, Liu Q, Xu W, Liang Y, Liu X, Li J, Yang R (2018). A new phthalife from the
577 endophytic fungus Xylaria sp. GDG-102. National Product Research. 32: 755-760.

578 36. Jiang CX, Li J, Zhang JM, Jin XJ, Yu B, Fang JG, Wu QX (2019). Isolation, identification, and activity
579 evaluation of chemical constituents from soil fungus Fusarium avenaceum SF-1502 and endophytic
580 fungus Fusarium proliferatum AF-04. J. Agriculture Food Chemistry. 67: 1839–1846.

581 37. Jouda JB, Tamokou JDD, Mbazoa CD, Douala-Meli C, Sarkar P, Bag PK, Wandji J (2016).
582 Antibacterial and cytotoxic cytochalasins from the endophytic fungus Phomopsis sp. harbored in
583 Garcinia kola (heckel) nut. BMC Complementary Alternative Medicine. 16(462).

584 38. Ondua M, Njoya EM, Abdalla MA, Lyndy J, Gaw M (2018). Anti-inflammatory and antioxidant
585 properties of leaf extracts of eleven South African medecinal plants used traditionally to treat
586 inflammation. Journal of Ethnopharmacology.

587 39. Hunter P (2012). The inflammation theory of disease. The growing realisation that chronic
588 inflammation is crucial in many diseases opens new avenues for treatment. European Molecular Biology
589 Organisation Rep. 13: 968-970.

590 40. Nairz M, Schleicher U, Schroll A, Sonweber T, Theurl I, Ludwiczeck S, Talasz H, Brandacher G,
591 Moser PL, Muckentaler MU, Fang FC, Bogdan C, Weiss G (2013). Nitric oxide-mediated regulation of
592 ferroportin-1 controls macrophages iron homeostasis and immune function of salmonella infection.
593 Journal of Experimental Medicine. 210(5) : 855-873.

594 41. Pechangou NS, Enang II EB, Ngohoba SV, Mfotie NE, Njayou FN, Moundipa FP (2022). Crude
595 Extracts of Codiaeum Variegatum stem exhibit potent antioxydant and anti-inflammatory activities in
596 vitro. Journal of Exploratory Research in Pharmacology. 000(000).

597