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Full Length Research Paper

Insignificant activity of *Allium paradoxium* and *Tanacetum parthenium* on protoscoleces of *Echinococcus granulosus*: In vitro study

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Medicinal plants are now recognized as most important alternative for chemical drugs resource, especially for anti-microbial targets. The current study aimed to determine the scolicidal effect of *Allium paradoxium* and *Tanacetum parthenium* as medicinal plants *in vitro*. Protoscoleces were exposed to different concentrations of chloroformic and hydroalcoholic extracts (1, 10, 50 and 100 mg/mL) for 5, 10, 30, 60 and 120 min. Viability of protoscolices was confirmed by 0.1% Eosin staining. The results of this study showed that all extracts used were not significantly effective in comparison with the control group. Therefore, chloroformic and hydroalcoholic extracts of *A. paradoxium* and *T. parthenium* could not be used during hydatid cyst treatment. However, there remains the need to perform advanced comparative clinical studies on the efficacy of *A. paradoxium* and *T. parthenium* and other scolicidal agents. Chloroformic and hydroalcoholic extract of *A. paradoxium* and *T. parthenium*, did not show any scolicidal activity at the concentration of 1, 10, 50 and 100 mg/ml.

Key words: *Allium paradoxium*, *Tanacetum parthenium*, *Echinococcus granulosus*, *in vitro*.

INTRODUCTION

Hydatid disease due to *Echinococcus granulosus* remains an imperative, challenging medical problem (Brunetti et al., 2010). It is endemic in developing regions because of their poor socioeconomic conditions, but because of travelling and migration, it continues to have a global distribution. Hydatid cysts may be found anywhere in the body, but liver and lungs are the most infected organs (Pawlowski et al., 2001). Surgery is still the chosen method of treatment (Topcu et al., 2006). Repetition during the long-term follow-up period is the most usual problem with this therapeutic technique. For decrease in

the recurrence rate of disease, it is necessary to avoid spilling of the cyst contents and the use of active scolicidal agents (Eckert et al., 2001). The World Health Organization (WHO) regards the use of both effective and safe agents for intraoperative killing of infectious material, but unfortunately; there is no ideal agent up to now. According to WHO reports, therapeutic plants with a relatively low risk are the suggested for traditional treatment of disease (WHO, 1996).

Allium paradoxium and *Tanacetum parthenium* are the Iranian native plants that indicate antimicrobial,

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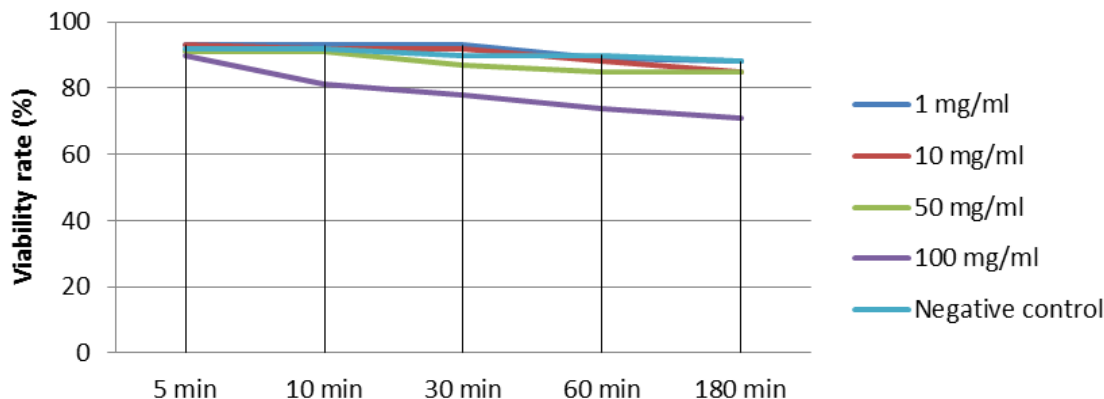


Figure 1. The effects of chloroformic extract of *A. paradoxium* on viability of protoscoleces of hydatid cyst in concentration of 1, 10, 50 and 100 mg/ml after 5, 10, 30, 60 and 180 min in comparison with the control group.

antifungal and antiviral effects. Also, these two plants showed anti-giardial effects *in vitro* (Shirzad et al., 2014). B-Pinene (6.45%), limonene (3.4%), Z-nerolidol (18.01%), spathulenol (22.06%), alpha-bisabolol (5.06%), phytol (9.15%), n-docosane (4.79%) and n-tricosane (3.8%) are the most important identified compounds of *A. paradoxium* (Akbar and Ahmadi, 2010). Camphor and chrysanthenyl acetate are the main components of flower heads of *T. parthenium* (Mostafa et al., 2007). Considering the low range of scolicidal compounds, searching for a new scolicidal agents, particularly from natural sources is of great interest (Rahimi-Esboei et al., 2012; Gholami et al., 2013).

Since *A. paradoxium* and *T. parthenium* has been shown to have a number of medicinal and anti-parasitic properties and considering the origin of these plants to Iran, the present study aimed to assess the scolicidal effect of the chloroformic and hydroalcoholic extracts at different concentrations and for various exposure times.

MATERIALS AND METHODS

Parasite

Hydatid cysts of *E. granulosus* were detached from livers of naturally infected sheep slaughtered at Mazandaran abattoir, northern part of Iran under aseptic conditions. The fluid of hydatid cyst containing protoscoleces was removed by a 50 ml syringe and transferred into a 50 ml Falcon tube. After 30 min of immovability, settled protoscoleces were washed three times with normal saline (pH 7.2). The viability of the parasites was assessed with 0.1% eosin under a light microscope (400x) (Gholami et al., 2013).

Preparation of extracts

The sliced seeds of *A. paradoxium* and the aerial parts of *T. parthenium* were dried under shade and powdered mechanically using a commercial electric blender. Dried powder (100 g) were extracted by percolation method with hydroalcohol and chloroform

solution, separately for 72 h in room temperature. The obtained solution was passed through filter paper (Whatman No.3, Sigma, Germany) to remove plant debris. Finally, the extracts were concentrated using a rotary evaporator (Heidolph, Germany), placed in a sterile glass and stored at -4°C for later use (Shirzad et al., 2014).

Scolicidal tests

The scolicidal tests were done three times (triplicate) and based on Rahimi-Esboei et al. (2013). In the current study, four concentrations (1, 10, 50 and 100 mg/ml) of extracts were examined for 5, 10, 30, 60 and 180 min. 2 ml of each concentration was transferred to a test tube; 50 µl of sediment protoscoleces was added to the tube and mixed lightly. The tube was then incubated at 37°C for 5, 10, 30, 60 and 180 min. At the end of each incubation time, viability of the protoscoleces was evaluated by 0.1% eosin stain (Rahimi-Esboei et al., 2013).

RESULTS

Results of the effectiveness of different concentrations of *A. paradoxium* and *T. parthenium* extract as a scolicidal agent are shown in Figures 1 to 4. While the viability rate in the control group was 88% after 180 min, scolicidal activity of chloroformic extract of *A. paradoxium* extract at concentration of 100 mg/mL was 90, 81, 78, 74 and 71% after 5, 10, 30, 60 and 180 min of application, respectively (Figure 1). Scolicidal effect of hydroalcoholic extract of *A. paradoxium* extract at concentration of 100 mg/mL was 88, 84, 79, 76 and 72% after 5, 10, 30, 60 and 120 min of exposure, respectively (Figure 2). While the viability rate in the control group was 85% after 180 min, scolicidal activity of chloroformic extract of *T. parthenium* extract at concentration of 100 mg/mL was 87, 85, 85, 81 and 78% after 5, 10, 30, 60 and 180 min of application, respectively (Figure 1).

Scolicidal effect of hydroalcoholic extract of *T. parthenium* extract at concentration of 100 mg/mL was

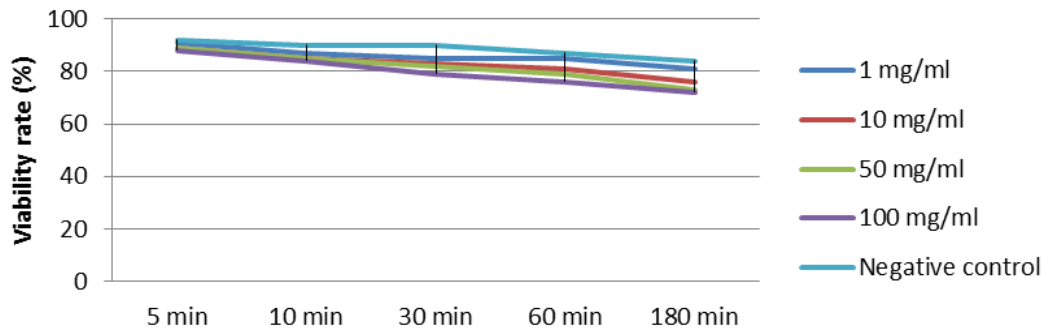


Figure 2. The effects of hydroalcoholic extract of *A. paradoxium* on viability of protoscoleces of hydatid cyst in concentration of 1, 10, 50 and 100 mg/ml after 5, 10, 30, 60 and 180 min in comparison with the control group.

Chart Title

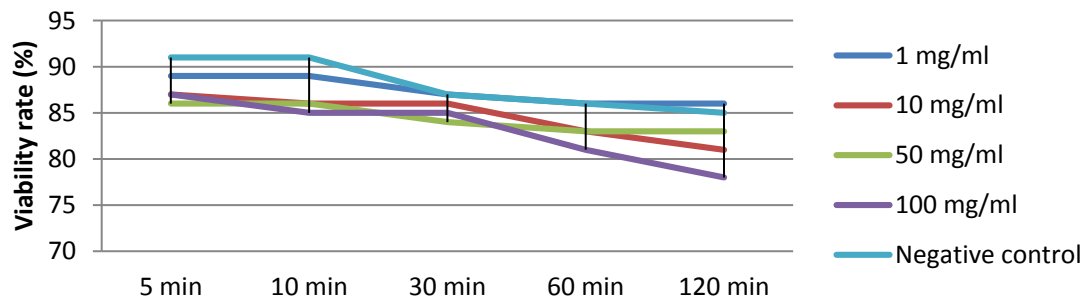


Figure 3. The effects of chloroformic extract of *T. parthenium* on viability of protoscoleces of hydatid cyst in concentration of 1, 10, 50 and 100 mg/ml after 5, 10, 30, 60 and 180 min in comparison with the control group.

84, 78, 78, 73 and 69% after 10, 20, 30 and 40 min of exposure, respectively (Figure 2).

In this study, the effectiveness of two plants were increased by increasing the time and concentration of plants, but there was no significant differences between times of exposure and concentration of extracts.

DISCUSSION

The current work examined the scolical activity of *A. paradoxium* and *T. parthenium* using the chloroformic and hydroalcoholic extracts and an *in vitro* technique. Shirzad et al. (2014) showed high effectiveness of *A. paradoxium* and *T. parthenium* on *Giardia lamblia* in *in vitro* investigation with concentration of 100 mg/ml after 3 h. Also, this anti-giardial activity of *A. paradoxium* was reported by Elmi et al. (2014) in an *in vivo* study. Despite the anti-bacterial and anti-parasitic activity of these plants, the results of this study indicate insignificant efficacy against protoscoleces of hydatid cyst (Shirzad et

al., 2014). It could be concluded that protoscoleces of hydatid cyst are more resistance than other used microorganisms and it depends on the physiological structure of cells. In this study, the used plants were inactive against protoscoleces of hydatid cyst. Perhaps, a longer exposition time was needed while the same concentration and exposure time was effective against some microbial and parasitic infections (Sarkari et al., 2009; Mehrabi et al., 2011; Radwan et al., 2012).

This *in vitro* technique is simple but confirmed, trustworthy, sensitive and extensively used (Dey, 1980). Therefore, the results gained are permissible and reliable. As shown (Figures 1 to 4), the chloroformic and hydroalcoholic extracts of *A. paradoxium* and *T. parthenium* did not exhibit any *in vitro* scolical activity, even at concentrations of 100 mg/ml after 180 min. From the extracts and plants, hydroalcoholic extract of *T. parthenium* was the most effective extract in concentration of 100 mg/ml after 180 min.

This study strongly recommends evaluating the activity with a longer period and develops more research on the

Chart Title

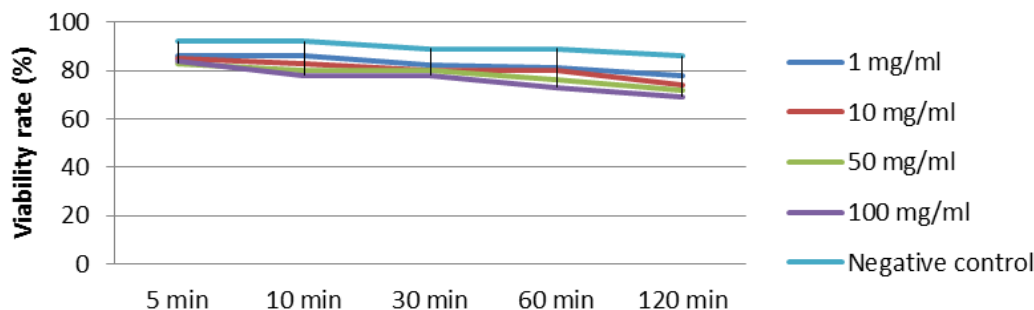


Figure 4. The effects of hydroalcoholic extract of *T. parthenium* on viability of protoscolexes of hydatid cyst in concentration of 1, 10, 50 and 100 mg/ml after 5, 10, 30, 60 and 180 min in comparison with the control group.

phytochemistry of these plants.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Antileishmanial effects of the alkaloid-rich fraction of *Quassia amara* L.

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Control of leishmaniasis remains a challenge due the high toxicity of the chemotherapeutic drugs presently available. The ongoing search for better leishmanicidal compounds has brought herbal drugs into the limelight as safe and effective substitutes to conventional therapies which have various drawbacks. The current study was designed to evaluate the antileishmanial effect of *Quassia amara* against *Leishmania amazonensis* and *Leishmania infantum*. Different fractions (Hexane (Q1), dichloromethane (Q2), ethyl acetate (Q3) and butanol (Q4)) obtained from the liquid-liquid partition of the crude *Q. amara* methanol extract were tested against *Leishmania* promastigote forms. After 120 h of treatment with *Q. amara* fractions (1 to 500 µg/ml), the minimal inhibitory concentration (MIC) was determined using resazurin. The most active fraction (Q2) was analyzed using thin layer chromatography (TLC) and high performance liquid chromatography-diode array detector (HPLC-DAD) techniques. The effects of Q2 on parasite ultrastructure were investigated by transmission electron microscopy. In addition, *Leishmania*-infected macrophages were treated with Q2 in order to evaluate the anti-amastigote effect. Among the fractions tested, Q2 showed the highest activity against *L. amazonensis* and *L. infantum* promastigotes MIC values of 62.5 and 31.25 µg/ml, respectively. There were ultrastructural alterations, such as nuclear chromatin condensation, intense vacuolization and autophagic and myelin-like figures in parasites treated with Q2 (62.5 µg/ml). Macrophages previously infected with *L. amazonensis* and *L. infantum* promastigotes showed a drastic reduction in the number of parasites recovered in the supernatant after Q2 treatment at MIC values. The TLC and HPLC fingerprints of Q2 showed that alkaloids were the main chemical constituents in this fraction. The results presented herein showed that the alkaloid-rich fraction Q2 is a promising source of antileishmanial agents. Further investigation will be necessary in order to isolate and test the substance(s) responsible for the bioactivity.

Key words: Antileishmanial activity, *Quassia* genus, alkaloids, macrophage infection, nitric oxide.

INTRODUCTION

Leishmaniasis is a group of illnesses caused by several species of protozoa belonging to the *Leishmania* genus. They are considered endemic in 98 countries and have a broad spectrum of clinical manifestations ranging from disfiguring skin lesions, known as tegumentary leishmaniasis (TL) to the visceral form of the disease, visceral leishmaniasis (VL) or kala-azar, that can lead to death (Hartley et al., 2012; Rebbestad et al., 2012). Brazil possesses the highest incidence of TL and VL with 26,008 and 3,481 cases reported, respectively (Roqueline et al., 2014). The dissemination of the disease may occur by human to-human transmission (anthroponotic transmission) or from animals to humans (zoonotic transmission). In both cases, parasites (infective metacyclic promastigote forms) are transmitted during the insect vector hematophagy (Chakravarty and Sundar, 2010). Once inoculated in the dermis-epidermis junction of the vertebrate hosts, infective parasites are phagocytized by macrophages and then initiate the differentiation process to amastigotes, the evolutive form responsible for the disease establishment and progression (De Pablos et al., 2016). Since macrophages play a pivotal role in the infection as host cells, the search for drugs able to elicit an effective antileishmanial response by these cells becomes essential.

Pentavalent antimony compounds and amphotericin B are the main drugs prescribed for the treatment of leishmaniasis. In addition, other drugs can be used such as pentamidine and paromomycin, which are more effective against the tegumentary form of the disease and miltefosine, the orally active drug indicated for VL treatment (Sundar and Singh, 2016; Dorlo et al., 2012; Frézard et al., 2009; Jhingran et al., 2009). However, these drugs present variable efficacies and high toxicity (e.g. cardiotoxicity, hepatotoxicity and nephrotoxicity), which may lead to diverse side effects. The development of parasite drug resistance is an additional problem for the control of leishmaniasis (Sundar and Chakravarty, 2015). In this scenario, several research laboratories around the world have been focused on the prospection of plant species as a strategy for discovery of more effective substances against leishmaniasis.

Quassia amara L. (Simaroubaceae) is a tree species found in tropical regions including Central and South America, where it is popularly known as “pau-tenente”, “amargo”, “grande hombre” or “quassia”. The tree can reach 8 m in height featuring compound leaves, red flower petals and drupes about 1 to 2 cm long. Several extracts obtained from the leaves, bark or wood of *Q. amara* have traditionally been used as natural remedies

for the treatment of digestive and liver disorders, and malaria (López and Pérez, 2008). In fact, the *Quassia* genus is a known source of biologically active extracts against protozoa. The aqueous extract of *Quassia africana* root bark displayed antitrypanosomal, anti-plasmodial and antileishmanial activities in concentrations ranging from 0.46 to 5.94 µg/ml (IC₅₀ values) (Musuyu Muganza et al., 2012). Some authors have described the antimalarial activity of *Q. amara* species. Ajaiyeoba et al. (1999) demonstrated that the methanol extract of *Q. amara* leaf displayed strong antimalarial activity in a murine model. Further investigations revealed that quassinoids such as simalikalactone D, simalikalactone E, quassin and nequassin were responsible for the antimalarial properties (Mishra et al., 2010; Cachet et al., 2009; Bertani et al., 2006). The present work investigates the antileishmanial potential of an alkaloid-rich fraction obtained from *Q. amara*.

MATERIALS AND METHODS

Plant and extraction procedures

Q. amara stem bark was commercially obtained in a popular street market located in Aracaju (Sergipe, Brazil). Samples were authenticated by Dr. Rosana C. Lopes at the RFA Herbarium, Department of Botany, Federal University of Rio de Janeiro (IB/UFRJ), where a voucher specimen is deposited (RFA 40769). The samples (315 g) were extracted for 5 days at room temperature using methanol (1 L). Then, the methanol extract was dried (yield =1.23%), resuspended in 150 ml of distilled water, and subjected to solvent partitioning (1:1) in the following order: hexane, dichloromethane, ethyl acetate and butanol. After the evaporation of the solvents Q1 (yield =20.5%), Q2 (yield =16.6%), Q3 (yield =2.96%) and Q4 (yield =0.87%) fractions were obtained, respectively, and kept in amber flasks at 4°C until biological and chromatographic analysis.

Thin layer chromatography (TLC) analysis

TLC was performed using TLC Silica gel plates (Merck), 0.25 mm; eluent system: 2% methanol/dichloromethane; detection: Dragendorff and sulfuric acid spray reagents. After spraying the plates were visually analyzed.

Q2 fraction analysis by fast high performance liquid chromatography-diode array detector (HPLC-DAD)

Liquid chromatography was performed using a Shimadzu fast HPLC-DAD. The sample was applied using an automatic injector and separated on a Kinetex C18 column, 150 mm × 4.6 mm, 2.6 µm at 35°C. The mobile phase was a gradient of solvent A (H₂O with 0.1% of formic acid) and B (acetonitrile) from 3 to 25% of B, 0

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to 12 min and from 25 to 75% of B, 12 to 22 min. The acetonitrile used was HPLC grade (Tedia) and the water was purified with a Milli-Q system. Q2 (2.0 mg) was dissolved in 1.0 ml of water and then centrifuged for 10 min prior the analysis. The flow and sample injection volume were 1 ml/min and 2.0 μ l, respectively. The chromatogram was acquired at a wavelength of 275 nm.

Parasites tested

Leishmania amazonensis (IFLA /BR/1967/PH8) and *Leishmania infantum* (MHOM/BR/1974/PP75) promastigotes were donated by the Leishmania Type Culture Collection of Oswaldo Cruz Institute/Fiocruz (Rio de Janeiro/RJ/Brazil). Parasites were axenically cultured in PBHIL medium supplemented with 10% fetal bovine serum (Rodrigues et al., 2010).

Leishmania inhibitory assay

In order to evaluate the antileishmanial activity of *Q. amara* extracts, *L. infantum* and *L. amazonensis* promastigote forms were harvest at early stationary phase (96 h), washed twice with PBS (150 mM NaCl; 20 mM phosphate buffer, pH 7.2) and re-suspended in fresh culture medium at a concentrations of 10^7 parasites/ml. Then, 100 μ l of the cellular suspension was added to 96-well microplates, where *Q. amara* fractions had been previously diluted (final concentrations ranging from 0.97 to 500 μ g/ml). The microplates were incubated at 28°C for 24, 48 and 120 h. Parasite growth was evaluated daily by direct counting of the cells in a Neubauer chamber. The minimum inhibitory concentration (MIC) was determined using resazurin as the cellular viability indicator (Rolon et al., 2006). Amphotericin B was used as positive control. Alternatively, cultures presenting negative parasite growth were re-incubated in fresh culture media aiming to evaluate a leishmanicidal or leishmanistatic effect.

Ultrastructure analysis

L. amazonensis promastigote forms were harvested at early stationary phase and treated with Q2 fraction at inhibitory (MIC) and sub-inhibitory (MIC/2) concentrations for 24 h at 28°C. After the incubation period, the parasites were washed twice with PBS and then fixed in 0.9% glutaraldehyde solution (2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer containing 3.5% sucrose, pH 7.4) at 4°C for 60 min. The samples were sent to the Rudolf Barth Platform (IOC/FIOCRUZ/RJ) and processed as previously described (Rodrigues et al., 2013). Image acquisition was made with a JEOL JEM1011 transmission electron microscope.

Cytotoxic evaluation

Peritoneal macrophages were obtained as previously described (Rodrigues et al., 2013). The maintenance procedures and euthanasia of animals were approved by the UFRJ Committee of Ethics for the Use of Animals (license 154/13). Macrophages (2.5×10^5 cells) were allowed to adhere into 96-well microplates for 20 min at 37°C and 5% CO₂ atmosphere. The macrophage cultures were washed with PBS and incubated in the presence of *Q. amara* Q2 fraction at concentrations ranging from 0.97 to 500 μ g/ml for 24 and 48 h. The cellular viability of Q2-treated macrophages was evaluated by resazurin reduction assay as previously described (Al-Musayeb et al., 2012).

Macrophage infection and nitric oxide production

The infection assays were performed following the protocol previously described by Passero et al. (2010) with slight modifications. Briefly, peritoneal macrophages (1.0×10^6 cells/ml) were distributed in 96-well microplates. Subsequently, promastigotes forms of *L. amazonensis* or *L. infantum* were added (5 parasites *per* macrophage). The parasite-macrophage interaction was performed in RPMI 1640 medium supplemented with 10% FBS at 37°C and 5% CO₂ atmosphere for 24 h. After the incubation period, free promastigotes were removed by extensive washing with PBS and then the cultures were treated with Q2 at 31.25 and 15.62 μ g/ml, MIC and subMIC, respectively. After 48 h of treatment, culture supernatants were collected for nitric oxide determination (Green et al., 1990). The infected macrophages were washed and re-incubated with PBHIL supplemented with 10% FBS for 72 h at 28°C in order to evaluate the number of promastigotes that recovered. The experiment was performed in triplicate and the number of viable promastigotes was determined by the average of cell counts using a Neubauer chamber.

Statistical analysis of the data

The mean and standard error of at least three experiments were determined. Statistical analysis of the differences between mean values obtained for experimental groups was done by means of Student's *t*-test. P values of 0.05 or less were considered significant.

RESULTS

Parasite growth inhibition

Among the four fractions, Q2, Q3 and Q4 displayed antileishmanial activity against *L. amazonensis* and *L. infantum* promastigotes with MIC values ranging from 31.25 to 500 μ g/ml (Table 1). Q1 was not active against the promastigotes at the highest concentration tested (MIC > 500 μ g/ml). Q2 presented the best results and was able to terminate *L. amazonensis* and *L. infantum* growth at 31.25 and 62.5 μ g/ml, respectively. Moreover, Q2 exerted an inhibitory effect on *Leishmania* growth at the sub-inhibitory concentrations (subMIC) of 15.62 and 31.25 μ g/ml for *L. amazonensis* and *L. infantum*, respectively (Figure 1A and 1B). Based on these results the researcher decided that all further experiments would be only with the Q2 fraction.

Ultrastructural alterations

Ultrastructural alterations in Q2-treated *L. amazonensis* promastigotes were investigated using TEM. Figure 2A and B shows control parasites displaying normal morphology and intracellular organelles. After 24 h exposure to 62.5 μ g/ml of Q2 (MIC value), significant alterations were observed in the parasites, such as electron dense granules and myelin-like figures in the cytoplasm (Figure C). In addition, parasites showed mitochondrial dilatation with concentric membranes in the

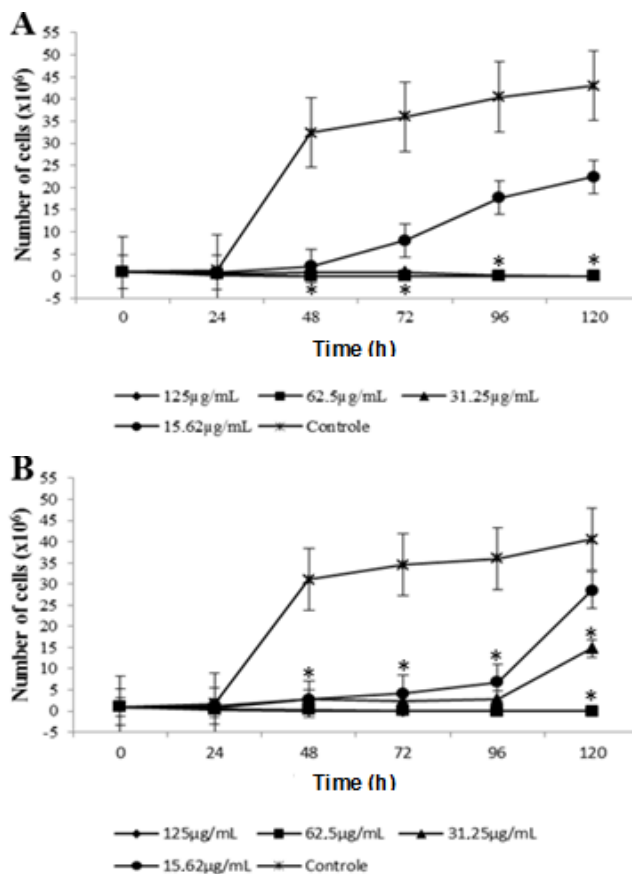


Figure 1. Antiproliferative effect of Q2 on *L. amazonensis* (A) and *L. infantum* (B) promastigotes. Parasites were counted at 24-h intervals using a Neubauer chamber. Each bar represents the mean \pm standard error of at least three independent experiments, which were performed in duplicate. The asterisks (*) indicate a significantly different value from the untreated parasites ($p < 0.05$).

mitochondrial matrix, nuclear chromatin condensation and the presence of autophagic structures (Figure 2E). Vesicles with cytoplasmic content released from the cell body into the flagellar pocket were also noted (Figure 2D).

Cytotoxicity for peritoneal macrophages

The effect of *Q. amara* Q2 fraction on peritoneal macrophages was investigated (Table 1). Q2 displayed toxic effect at 500 $\mu\text{g/ml}$ (minimal cytotoxic concentration - MCC); allowing its use at effective concentrations against *L. amazonensis* and *L. infantum*.

Anti-amastigote activity

In order to evaluate the effects of Q2 *in vitro*, peritoneal macrophages were previously infected with *L. amazonensis* or *L. infantum* promastigotes and then

treated with Q2 at MIC and subMIC concentrations. After 48 h of infection, Q2 was removed by washing and the cultures were reincubated under proper conditions for promastigote recovery. The results demonstrate that the number of promastigotes recovered in the treated macrophage culture supernatants was very low, regardless of the Q2 concentration tested (Table 2).

In addition, the nitric oxide (NO) produced by the macrophages during the infection period was determined. The study showed that only *L. amazonensis*- and *L. infantum*-infected macrophages treated at MIC concentration of Q2 produced high amounts of NO when compared to their respective untreated cultures. Indeed, the NO production was about 159 and 183% higher than the untreated cultures, respectively.

Chromatographic analysis of Q2

Alkaloid-rich fraction (Q2) was detected by TLC with

Table 1. Antileishmanial activity of the four fractions derived from the *Q. amara* methanol extract.

Fractions	Minimal inhibitory concentrations ($\mu\text{g/ml}$)					
	<i>L. amazonensis</i>			<i>L. infantum</i>		
	24 h	48 h	120 h	24 h	48 h	120 h
Q1	>500.0	>500.0	>500.0	>500.0	>500.0	>500.0
Q2	62.5	62.5	31.25	125.0	62.5	62.5
Q3	500.0	62.5	62.5	>500.0	250.0	250.0
Q4	250.0	125.0	125.0	>500.0	500	500
AmphB	1.95	0.97	0.97	0.97	0.48	0.48

Q1: *Q. amara* hexane fraction; Q2: *Q. amara* dichloromethane fraction; Q3: *Q. amara* ethyl acetate fraction; Q4: *Q. amara* butanol fraction; AmphB: Amphotericin B.

Table 2. Effects of Q2 on *L. amazonensis* and *L. infantum* intracellular amastigotes and nitric oxide production by infected macrophages.

Q2 treatment	Promastigote recovery ($\times 10^4$ cells/mL)		NO_2 (μM)	
	<i>La</i> ($\pm\text{SE}$)	<i>Li</i> ($\pm\text{SE}$)	S_{La} ($\pm\text{SE}$)	S_{Li} ($\pm\text{SE}$)
Control	46.0 (± 2.55)	33.0 (± 6.33)	2.03 (± 0.3)	6.23 (± 2.0)
MIC	0.01 (± 5.47)	0.04 (± 0.03)	5.75 (± 0.3)	16.2 (± 3.3)
SubMIC	0.01 (± 3.92)	0.17 (± 0.1)	3.14 (± 0.8)	6.37 (± 3.0)

La: *Leishmania amazonensis*; *Li*: *Leishmania infantum*; S_{La} : Supernatant of *L. amazonensis*-infected cultures; S_{Li} : Supernatant of *L. infantum*-infected cultures; MIC: minimum inhibitory activity; SubMIC: sub-inhibitory concentration. MIC and subMIC values for *L. amazonensis* were 31.25 and 15.62 $\mu\text{g/ml}$, respectively. MIC and subMIC values for *L. infantum* were 62.5 and 31.25 $\mu\text{g/ml}$, respectively.

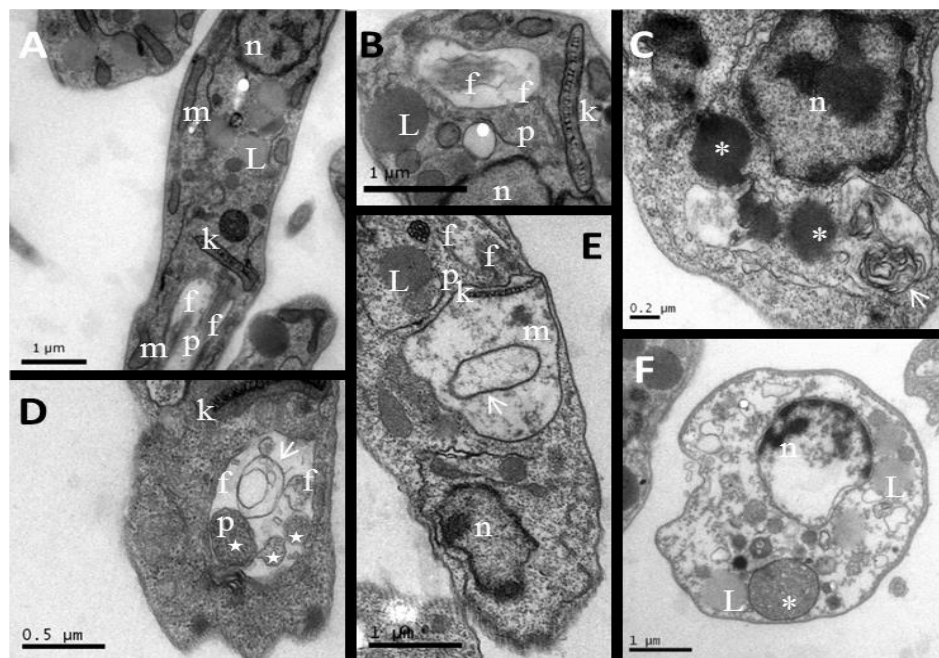


Figure 2. Photomicrographs of *L. amazonensis* promastigotes treated with Q2 fraction. (A-B) Control. (B) Flagellar pocket and mitochondrion containing the kinetoplast. (C-F) MIC-treated parasites. (C) Nuclear chromatin condensation, electron dense bodies (*) and myelin-like figures (L). (D) Dilated flagellar pocket containing vacuoles (*) and a multi vesicular structure (L). (E) Mitochondrial swelling and a vesicle (L) inside this organelle. (F) Intense vacuolization process and an autophagic structure (*). n, nucleus; m, mitochondrion; k, kinetoplast; f, flagellum; fp, flagellar pocket; L, lipid.

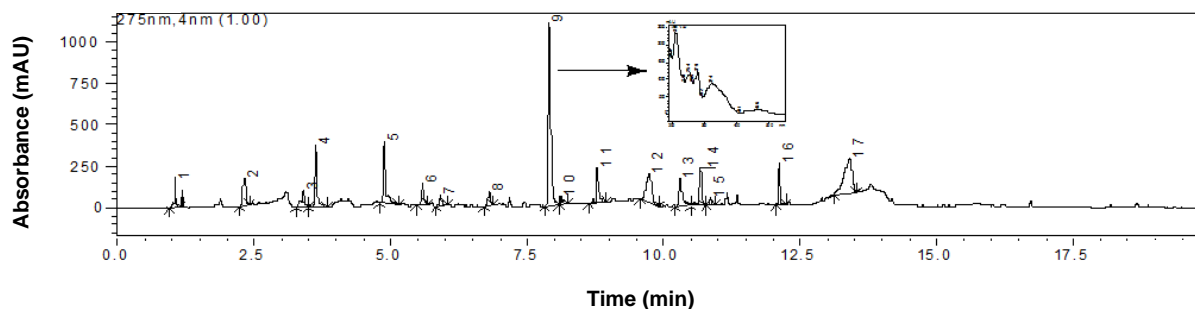


Figure 3. HPLC-DAD chromatogram of Q2 and UV spectrum of the main alkaloid.

characteristic orange color after revelation with Dragendorff reagent. The spots on TLC plates did not show red coloration when sprayed with sulfuric acid reagent which indicates the probable absence of quassinoids (Polonsky et al., 1980). The chemical fingerprint obtained by the HPLC-DAD analysis confirmed the presence of this class of substances, showing their characteristic UV spectra (Figure 3).

DISCUSSION

Plants represent a promising source of medicinal substances that are biologically active against several pathogens, including *Leishmania*. Sarkar et al. (2013) reported that the antileishmanial activity of the petroleum ether, chloroform and methanol extracts obtained from the leaves of *Pleumeria pudica*. In that study, all the extracts had strong activity against the *Leishmania donovani* promastigote forms with IC_{50} values ranging from 2.93 to 3.04 $\mu\text{g/ml}$. Triterpenoids were the main substances found in the extracts and may be responsible for the bioactivity (Sarkar et al., 2013). The crude methanol extract of *Lantana ukambensis* leaves was also active against *L. donovani* with IC_{50} value at 6.9 $\mu\text{g/ml}$ (Sawadogo et al., 2012a). Tannins, triterpenes, and steroids were previously reported by these authors as the main substances of the extract (Sawadogo et al., 2012b). Bhattacharjee et al. (2009) studied the effect of the quassinoid quassin isolated from *Q. amara* as immunomodulator for controlling the establishment of *L. donovani* infection. The use of *Q. amara* against different infectious diseases (Mans et al., 2016; Bertani et al., 2006; Ajaiyeoba et al., 1999) encouraged us to investigate the antileishmanial potential of this species against *L. amazonensis* and *L. infantum*.

Using the bioguided approach, the study reported that Q2 (dichloromethane fraction of the crude methanol extract) was the most active fraction against both parasites after treatment for 120 h. Table 1 shows that the antileishmanial activity of the extracts, including Q2, is time- and dose-dependent. Other species belonging to

the Simaroubaceae family have been described as biologically active against several pathogens in a time and dose dependent manner. Gu et al. (2014) reported that the ethanol extract from *Ailanthus altissima* increased *Psoroptes cuniculi* and *Sarcoptes scabiei* mortality with increasing both extract concentration and post-treatment time. In the present study, MIC values decreased in one fold dilution when parasites were treated for 120h. Parasite killing was confirmed after reincubation of inhibited cultures in fresh medium and no growth was detected. In addition, Figure 1 shows that *L. amazonensis* promastigote growth was strongly inhibited by Q2 in the first 72 h of treatment even at the sub-inhibitory concentration of 15.62 $\mu\text{g/mL}$. The complete elimination of the parasites occurred after 96 h of treatment at 31.25 $\mu\text{g/ml}$. On the other hand, the growth of *L. infantum* promastigotes was inhibited by all Q2 concentrations tested until 96 h of treatment when compared with control cells. However, parasites regained growth after this period except those treated at concentrations above 62.5 $\mu\text{g/ml}$. In order to determine if Q2 displays leishmanicidal activity, the *L. amazonensis* and *L. infantum* promastigote forms treated with MIC values (31.25 and 62.5 $\mu\text{g/mL}$, respectively) were re-introduced into fresh culture medium (in the absence of Q2). We observed that those cultures were no longer able to grow, showing strong evidence that Q2 displayed a leishmanicidal effect.

Q2 caused serious damages to the *L. amazonensis* promastigote forms. Mitochondrial swelling, the presence of myelin-like figures and autophagosomal structures on cytoplasm suggested disturbances of the sterol biosynthesis (Figure 2). This study is in line with other study report when using known or novel synthetic ergosterol synthesis inhibitors to treat *Leishmania* (de Macedo-Silva et al., 2015; de Macedo-Silva et al., 2013; Zeiman et al., 2008). Plant-derived products, extracts or isolated substances can inhibit *Leishmania* sterol biosynthesis. Previously, our group reported that a fraction (B2) obtained from the hexane extract of *Arrabidaea chica* caused similar damages to *L. infantum* promastigotes (Rodrigues et al., 2014). *L. amazonensis*

treatment with 1 to 5 μM tomatidine, a tomato alkaloid, led to the inhibition of $\Delta 24(25)$ -sterol methyltransferase, an essential enzyme for the ergosterol production (Medina et al., 2012).

Several studies have shown that alkaloids, flavonoids, chalcones, terpenoids, saponins, and phenolic compounds display antileishmanial effect (Sandjo et al., 2016; Singh et al., 2014; Wink, 2012). In the present study, HPLC-DAD was employed to evaluate the phytochemical profile of Q2. This technique is able to define the chemical classes of substances from natural origins based on their characteristic UV spectrum (Escarpa and Gonzalez, 1998; Justesen et al., 1998). The principal substances of Q2 detected by TLC presented orange spots after chemical revelation with Dragendorff reagent and HPLC/DAD demonstrated the UV spectra that indicate alkaloid class (Figure 3). There are few studies on the alkaloid content of *Q. amara*, which includes indole and canthin-6-one alkaloids (Barbetti et al., 1987; Barbetti et al., 1990; Njar et al., 1993). In fact, the investigation of the pharmacological potential of *Q. amara*-isolated alkaloids has been neglected, since quassinoids have been described as the main biologically active agents in this species. In this study, Q2 fraction rich in alkaloids displayed minimum cytotoxic concentration against peritoneal macrophages at 500 $\mu\text{g}/\text{ml}$. In addition, Q2 reduced the number of parasites recovered on *L. amazonensis*- and *L. infantum*-infected macrophages cultures at MIC concentrations, with a concomitant increase of NO production of about 2.8 and 2.6 fold higher than the controls, respectively (Table 2). Recently, Mans et al. (2016) reported the antileishmanial activity of 25 Surinamese medicinal plants traditionally used to treat cutaneous leishmaniasis, including *Q. amara*. The authors observed that the *Q. amara* aqueous extract was able to inhibit *L. major* NADIM5 and *L. donovani* GEDII promastigotes growth (IC₅₀ values of 51 and 68 $\mu\text{g}/\text{ml}$, respectively). However, the aqueous extract of *Q. amara* was extremely toxic for THP-1 cells (IC₅₀ < 16 $\mu\text{g}/\text{ml}$). They also observed that the aqueous extract reduced in 50% the number of intracellular amastigotes at 288 $\mu\text{g}/\text{ml}$. These data is controversial since cytotoxic concentrations were used in the infection assay. In the present study, infected macrophages were treated with parasite subinhibitory concentrations, allowing us to evaluate the treatment effect over internalized parasites without macrophage damage. Nevertheless, the study cannot assure that the obtained results were caused by the alkaloid content of Q2. Studies in our laboratory are in progress in order to fractionate Q2 and evaluate the antileishmanial activity of the isolated substances.

Conclusion

Our results demonstrated the antileishmanial properties of *Q. amara* against the etiological agents of tegumentary and visceral leishmaniasis, *L. amazonensis* and *L.*

infantum, respectively. Despite the quassinoids bioactivity described in the literature, the study showed that an alkaloid-rich fraction (Q2) obtained from *Q. amara* was the most active fraction against the parasites. This is an interesting finding due the relevance of alkaloids as potential therapeutic agents. The present study highlights *Q. amara*, a plant widely used in folk medicine, as a source of antileishmanial agents.

Conflict of interests

The authors have not declared any conflict of interest.

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Full Length Research Paper

In silico* physico-chemical evaluation, anti-inflammatory and mcf-7 breast cancer cell line growth inhibition effects of trolline isolated from *Mirabilis jalapa

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The *in silico* simulations and predictions approach in evaluation of pharmacokinetic properties of new chemical entities (NCEs) is fast becoming an acceptable trend in natural products research and drug discovery. This paper focuses on the properties of trolline with respect to its anti-inflammatory and MCF-7 breast cancer cell line growth inhibition effects and an attempt to predict physico-chemical drug-like properties *in silico*. The compound was isolated for the first time from aerial parts of *Mirabilis jalapa* and the structure was elucidated by 1D and 2D NMR, FTIR and mass spectroscopic analyses. The compound was screened for anti-inflammatory and anti-MCF-7 cell lines proliferation using chemoluminescence oxidative burst and MTT assays respectively. Predictions on physico-chemical properties central to oral route drug administration were done using commercial software Admet predictor. Results show anti-inflammatory effects at IC₅₀ concentration of 53.8 µg/ml and 48% mortality of breast cancer cell lines at 50 µM concentration. Predictions suggest moderate solubility of 1.72 to 2.92 mg/ml across pH range of 1.6 to 6.8 in simulated solvent systems and effective jejunal permeability of 5.61 x 10⁻⁴ cm/s. The compound was also predicted to be 60% unbound to plasma proteins and an LD₅₀ of 950 mg/kg in rats. The compound, trolline had clinically important bioactive effects, probably possess promising drug-like properties suitable for further high throughput screening.

Key words: ADMET, anti-inflammatory, *in silico* prediction, *Mirabilis jalapa*, NMR, trolline.

INTRODUCTION

The search for new chemical entities (NCEs) has prompted the resurgence in isolation and screening of natural products from diverse sources. Although, the last decade experienced a decline in the interest in natural

products research, this has not necessarily resulted in increase in NCEs approved as drugs through combinatorial chemistry aided synthesis (Harvey, 2008). So far, the best-selling therapeutic drugs as at 2012 are

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natural products or derived from them (Newman and Cragg, 2012). Quite a number of medicinal plants and other sources of natural products like the actinomycetes have been identified and are currently researched for their potential as sources of lead candidates for NCEs that are of clinical importance.

The plant, *Mirabilis jalapa* Linn. belongs to the *Nyctagenaceae* family, a perennial herbaceous plant that grows to a height of 50 to 100 cm. The seeds are wrinkled and turn black upon maturity while its beautiful flowers open around 4 O' clock (Nair et al., 2005). The plant commonly called marvel of Peru in English is indigenous to South America and other tropical climates (Taylor, 2005). In Nigeria, it is locally known as *Tonaposo* in Yoruba language (Elufioye et al., 2012) while in Pakistan, it is called *Gul-abbas* (Dhar et al., 1968). Phytochemical investigation reveals the presence of β -sitosterol, ursolic acid, amino acids, β -stigmaterol (Singh and Mittal, 2012), phytol (Siddiqui et al., 1994) in the aerial parts while rotenoids (Xu et al., 2010) and trigonelline (Zhou et al., 2012) alkaloid have been characterized from the roots. The plant is known to possess medicinal properties across different cultures. In Brazilian folk medicine for example, the leaf extract is used in treatment of inflammation (Walker et al., 2008). Extracts of the aerial parts are also used as purgative and in the treatment of dysentery (Encarnacion et al., 1998) and diarrhea (Holdsworth, 1992; Comerford, 1996). In Chinese traditional medicine, the root of this plant is used as an anti-diabetic (Zhou et al., 2012). Several literature reports on scientific studies on various parts of the plant suggest the presence of clinically important pharmacologic properties (as claimed), including antibacterial (Eneji et al., 2011), anti-viral (Wong et al., 1992), anti-inflammatory (Nath et al., 2010) and treatment for dysentery (Shaik et al., 2012). Importantly, bioguided isolation of small molecules from *M. jalapa* have also been demonstrated to have clinically important properties including trigonelline (Zhou et al., 2012) and boravinone rotenoids (Xu et al., 2010) isolated from the roots of this plant were shown to have anti-diabetic and in vitro anti-cancer activities, respectively.

The cost of bringing a new drug into the market usually requires heavy budgetary commitments from pharmaceutical industries. Indeed many lead compounds have either been rejected or fail completely at advanced stages of clinical trials. More complicating is the withdrawal of otherwise approved drugs from the market due to evidences emerging from pharmacovigilance (McNaughton et al., 2014; Onakpoya et al., 2015). Developments in *in silico* simulations and predictions using reliable data obtained from laboratory experiments have proven to provide the platform for the advancement of research in screening and testing of NCEs (Yu and Adedoyin, 2003; Moroy et al., 2012) as a viable alternative to circumvent these problems that result in huge investments go down the drain. There are

commercially available softwares (Boobis et al., 2002) that predict and simulate the fate of drug components as they interact with different compartments of the body notably, the administration, distribution, metabolism, elimination and toxic (ADMETox) outcomes of NCEs. *In silico* models built around the quantitative structure activity relationship (QSAR) concepts for example, have helped describe physicochemical properties of molecules by reliable laboratory data parameterization and development of molecular descriptors (Butina et al., 2002).

A careful study of reported activities of *M. jalapa* suggest constituents of polar fractions of the various plant parts may contain substances responsible for observed activities (Muthumani et al., 2010; Nath et al., 2010; Oladunmoye, 2012). The isolation of 8,9-dihydroxy-1,5,6,10b-tetrahydropyrrolo[2,1-a]isoquinolin-3(2H)-one (trolline) from polar fractions of aerial parts of *M. jalapa* is reported. This compound was previously isolated from the plants *Salsola collina* Pall (Zhao and Ding, 2004) and *Trollius chinensis* (Li-Jia et al., 2014). The following study seeks to investigate possible anti-inflammatory properties and the ability of this compound to inhibit growth of MCF-7 breast cancer cell lines *in vitro*. The physicochemical drug-like properties of this compound were also evaluated by *in silico* predictions.

MATERIALS AND METHODS

Aerial parts of *M. jalapa* L. plant was collected from the National Veterinary Research Institute (NVRI) Vom, Plateau State Nigeria. A voucher number 2441 was deposited having been identified at the herbarium section of the Biological Science Department, Ahmadu Bello University Zaria, Nigeria. The plant parts were air dried under shade and then crushed using pestle and mortar. The coarse particles size material was then immediately extracted using organic solvent.

Plant extraction and chromatography

Methanol was used to extract 29 g of pulverized plant parts after which hydrated butanol was used to re-extract by partitioning. Fractions F50-F80 obtained from silica column (mesh size 60-120) eluted with 10% MeOH : DCM were pulled together and further separated on flash column, 40% acetone : hexane elution. Fractions F33-F45 from this column were pulled and separated on MCI-gel column eluted with 60% H₂O : MeOH to obtain F23. The fraction F23 was further purified using recycling HPLC (JAI, RP column ODS H-80) at a flow rate of 2.5 ml/min to obtain 12 mg of the pure compound. The sonicated HPLC grade isocratic solvent system used was 60% H₂O : MeOH.

Spectroscopic data

IR (3425, 2925, 1649, 1527, 1452cm⁻¹); ¹H NMR (600MHz, MeOD) δ 6.55-6.53 (s, 2H), 4.72 (dd $J=$ 18.0, 7.8, 1H), 4.08 (ddd, $J=$ 12.9, 6.0, 2.8, 1H), 3.04 (td, $J=$ 12.0, 4.3, 1H), 2.76-2.70 (m, 1H), 2.66-2.60 (m, 2H), 2.56 (dd, $J=$ 17.9, 7.9, 1H), 2.41-2.36 (m, 1H), 1.80-1.73 (m, 1H); ¹³C NMR (151MHz, MeOD DEPT) C 175.9, 145.6,

Table 1. NMR spectroscopic data for trolline (600 MHz, MeOD).

Position	δ_c type	δ_H (J in Hz)	HMBC ^a
1	28.8 CH ₂	1.83-1.73 m 2.66-2.60 m	2, 4, 10b
2	32.7 CH ₂	2.56, dd (17.9,7.9)	1, 3, 4
3	175.9 C		
4	58.3 CH	4.72, dd (18.0, 7.8)	1, 6b, 10b
5	38.6 CH ₂	4.08, ddd (12.9, 6.0, 2.8) 3.04, td (12.0, 4.3)	3, 4, 6, 6b
6	28.8 CH ₂	2.76-2.70 m 2.66-2.60 m	5, 6b, 10b
6b	125.5 C		
7	116.2 CH	6.55-6.53 s	6, 9, 10b
8	145.5 C		
9	145.6 C		
10	112.4 CH	6.55-6.53 s	4, 6b, 8
10b	129.8 C		

^aProton-carbon HMBC for 2-3 bond connectivity.

145.5, 129.8, 125.5; CH 116.2, 112.4, 58.3; CH₂ 38.6, 32.7, 28.8, 28.8; HREIMS M⁺ 219.0882 Molecular formula C₁₂H₁₃NO₃; Ring plus double bond (RDB) analysis =7.

In vitro biological screening

Anti-inflammatory assay

Chemoluminescence method as described by Helfand et al. (1982) was adopted. Briefly, 25 μ L from each of the prepared trolline concentrations of 1, 10 and 100 mg/ml was incubated with 25 μ L of diluted whole blood HBSS⁺⁺ (Hanks Balanced Salt Solution, containing calcium chloride and magnesium chloride) [Sigma, St. Louis, USA]. Incubation of prepared cells and control (containing no test compound) was done at 37°C for 15 min in white half area 96 well plates (Costar, NY, USA) in a luminometer thermostat chamber (Labsystems Helsinki, Finland). Exactly 25 μ L each of opsonized zymosan (Fluker Buchs, Switzerland) and luminol (Research Organics Cleveland, USA) were added into each well except for blank wells containing only HBSS⁺⁺. Readings indicating reactive oxygen species (ROS) levels were recorded in luminometer and expressed as relative light units (RLU). Ibuprofen was used as standard control anti-inflammatory drug.

MCF-7 breast cancer cell lines MTT assay

The method as described by Scudiere et al. (1988) involves seeding 96 well tissue culture treated flat bottom plates with precultured MCF-7 cell lines in Dulbecco's modified Eagle medium (5% CO₂ incubator at 37°C). After incubation for 24 h, 50 μ M of trolline was added to each well in triplicate and incubated for 48 h. The tetrazolium salt MTT (3-(4,5-dimethylthiazol-yl)-2,5-diphenyl tetrazolium bromide) was added (200 μ L) after washing the cells and then incubated for 3 h at 37°C. Formazan crystals formed were dissolved in 100 μ L DMSO and absorbance taken at 570 nm using micro-plate reader (Spectra Max plus, Molecular devices, CA, USA). Results were recorded as percent growth inhibition of viable cells. Prepared alongside was doxorubicin as the control drug.

In silico evaluation

The physicochemical properties of trolline were evaluated using Admet predictor software, a commercially available licensed property of Simulations plus Inc Lancaster, USA. SMILE notations of isolated compound and controls used in the assays described was used to create the input files. Predictions were carried out on the 2D generated structures of the compounds using default settings of pH 7.4.

RESULTS

NMR assignments

As presented in the spectroscopic data, the molecular formula as determined from HREI-MS is C₁₂H₁₃NO₃ with pseudo molecular ion peak appearing at 218 m/z. The molecular mass was confirmed by ESI-MS with [M+H]⁺ 220 m/z. The FTIR analysis is in conformity with the odd numbered molecular mass suggesting the presence of N atom occurring as a tertiary amide functional group. NMR ¹³C (DEPT) analysis identifies presence of 12 carbons defined thus, 5 quaternary (1 carbonyl and 4 aromatic carbons), 3 methine (2 quaternary and 1 aliphatic) and 4 aliphatic methylene carbons. Presented in Table 1 is the NMR assignment using 2D spectroscopic data. The aromatic methine protons are assigned to C7 (δ 116.2) and C10 (δ 112.4) in *para* positions due to the tall singlet observed in ¹H NMR. The HMBC experiment (Table 1) described in the schematic (Figure 1) shows the 2-3 bond connectivity.

Bioactivities

Figure 2 presents the bioactive effects of trolline on anti-

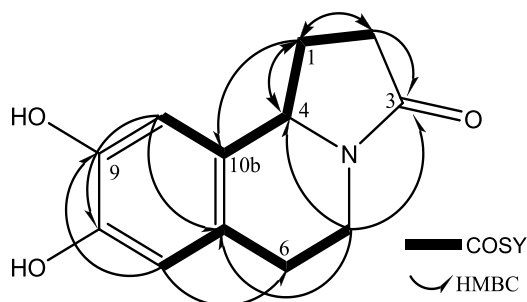


Figure 1. HMBC correlations ($^1\text{H} - ^{13}\text{C}$) for the compound, trolline.

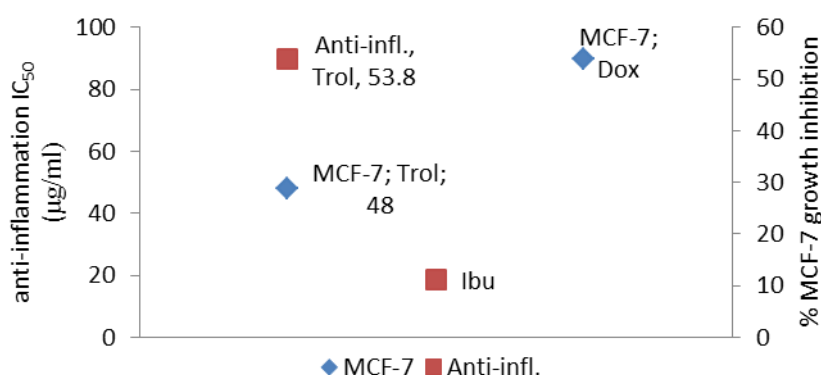


Figure 2. Anti-inflammation and anti-MCF-7 proliferative effects of trolline.

inflammation and growth inhibition of MCF-7 breast cancer cell lines. The results show IC₅₀ of 53.8 µg/ml concentration on anti-inflammatory effects and 48% inhibition of MCF-7 cell lines proliferation at 50 µM.

In silico predictions

Predictions on solubility in different solvent systems as presented in Figure 3 indicate that trolline is moderately soluble in gastric and intestinal fluids. Prediction models suggest the compound to be soluble in aqueous environment over a pH range of 1.6 to 6.8. Models for permeability predictions (Figure 4) indicate 5.61×10^{-4} cm/s effective jejunal permeability for trolline which is relatively high as compared to the cytotoxic drug doxorubicin predicted to have $<0.5 \times 10^{-4}$ cm/s, suggesting a low permeability property for this compound.

Plasma protein binding properties of trolline presented in Table 2 indicate the compound exist more in the free state rather than bound to plasma proteins. Trolline preferentially sequester within the blood rather than plasma (RBP = 1.25) and has minimal tissue distribution (as the model predicts).

DISCUSSION

The concentration of 53.8 µg/ml (0.25 mM) recorded for anti-inflammation could be considered significant as it falls within minimum concentration (IC₅₀) of 156 to 400 µg/ml (Choudhary et al., 2009). Inflammation is a normal physiologic response to disease and tissue damage. There are emerging evidences however, linking chronic inflammation and carcinogenicity (Coussens and Werb, 2002; Coussens et al., 2013), notably the chemokine system and recruitment of tumor related macrophages (Mantovani et al., 2010). Although, 90% mortality was recorded for doxorubicin at 50 µM (Figure 2), non-target cells cytotoxicity and other contra-indications associated with use of doxorubicin are limitations to the use of this compound (Alexieva et al., 2014). Comparatively however, and at similar concentrations, trolline was observed to inhibit 48% growth of MCF-7 breast cancer cells. Compounds with pyrrolo[2,1-a]isoquinoline structure are reported to have hypotensive and anti-tumor activities (Mikhailovskii and Shklyayev, 1997) as well as antibacterial properties (Wang et al., 2004). Oleracein E (the antipode of trolline) has been reported to exhibit potent DPPH radical scavenging activity (Yang et al., 2009). Other cytotoxic compounds characterized from *M.*

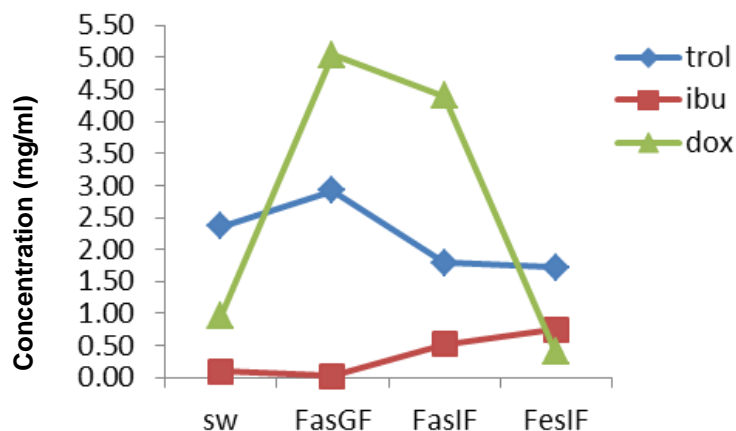


Figure 3. Prediction models on compound solubility. Sw = native solubility in water; FasGF = solubility in simulated fasted state gastric fluid; FasIF = solubility in simulated fasted state intestinal fluid; FeSIF = solubility in simulated fed state intestinal fluid; Simulations Plus.

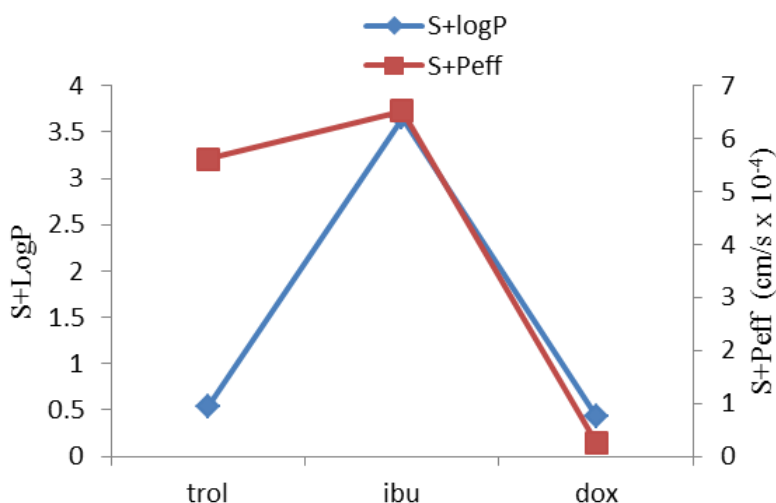


Figure 4. Prediction models on jejunal permeability. S+logP = octanol-water partition coefficient; s+Peff = human jejunal effective permeability.

Table 2. Predictions on protein binding, volume of distribution and blood-plasma concentrations.

Compound	PrUnbnd (%)	VD (L/Kg)	RPB
Trolline	60.23	0.95	1.25
Ibuprofen	2.10	0.34	0.58
Doxorubicin	21.56	11.48	0.99

PrUnbnd: % unbound proteins; VD: volume of distribution; RPB: blood-plasma ratio.

jalapa, notably the cytotoxic rotenoids mirabilalones and boeravinones have been shown to have growth inhibition

effects against several cell lines (Wang et al., 2002; Xu et al., 2010).

Predictions on absorption properties for trolline conforms with laboratory findings using human Caco-2 cell monolayer model (Li-Jia et al., 2014), suggesting trolline to be moderately absorbed in the GIT. Similar trends in prediction results were observed for ibuprofen used as control for anti-inflammation properties; shown to be highly lipophilic and permeable to the jejunum (Figure 4) but relatively low in solubility (Figure 3). Ibuprofen traversing the GIT is a pH dependent phenomenon, permeable through walls of the stomach but not readily soluble; thus, limiting its entry into systemic circulation (Patel et al., 2011).

Table 3. Predictions on compound LD₅₀ and TD₅₀ in rats.

Compound	LD ₅₀ (mg/Kg)	TD ₅₀ (mg/Kg/day)
Trolline	950.00	107.23
Ibuprofen	1175.37	82.27
Doxorubicin	463.14	19.17

LD₅₀ = LD50 for lethal rat acute toxicity; TD₅₀ = TD50, defined as the oral dose of a compound required to induce tumors in 50% of a rat population after exposure over a standard lifetime.

Key parameters for drugs intended for oral administration are solubility and permeability. Dissolution of target drug dose around site of absorption increases the propensity for bioavailability (Amidon et al., 1995). Thus, early identification of low solubility drug candidates estimated at 40% of new drugs (Dahan and Muller, 2012) is important in the long term downstream screening. High jejunal permeability was predicted for trolline and was also shown to be present in blood more in the free state rather than bound to plasma proteins. Model prediction on the control drug doxorubicin (Table 2) however indicates high volume of tissue distribution, which is consistent with literature reports (809-1214 Lm⁻²) on the pharmacokinetics of doxorubicin with a distribution half-life of 5 min (National Center for Biotechnology Information, 2016).

Existence of drugs in free unbound state allows for effective interactions with target receptors thus increasing drug efficacy. Alterations in plasma protein binding inevitably affect dose formulations, clearance time and systemic distribution of drugs (Scheife, 1989; Roberts et al., 2013). The quantitative structure activity relationships (QSAR) methods used in developing descriptors for *in-silico* predictions provide insights into the probable behaviour of NCEs with plasma proteins (Saiakhov et al., 2000; Ghafourian and Amin, 2013).

Communities, especially in developing countries where use of medicinal plant is a common cultural practice, these plants are often not regarded as food but are however consumed at concentrations exceeding what is obtainable in clinical practice. Predictions (Table 3) on lethal rat acute toxicity (LD₅₀) observed for trolline was 950 mg/kg; and a life time oral dose administration (TD₅₀) of 107.23 mg/kg/day is likely to induce tumors (in rats). Considering standard practice in drug dosage formulations, we assume from predicted values as well as moderate toxicity against MCF-7 breast cancer cells (*in vitro*), trolline may not constitute a threat to normal body homeostasis but construed to play functional roles in the long term prevention of early onset of disease and other forms of tissue damage. However, this is a subject matter for clinical verification. *In silico* predictions on administration, distribution, metabolism and elimination (ADME) of drugs in modern day research has the advantage of limiting time and reducing the cost of searching for NCEs with drug-like potentials (Butina et

al., 2002; Kapetanovic, 2008).

Conclusion

In silico simulation and prediction are becoming more efficient and fast gaining popularity and robust applicability in pharmaceutical research. The development of proficient machine language programming has revolutionized this tool as a necessary cost effective step in drug discovery. The compound, trolline was demonstrated to have clinically important bioactive properties and predictions on the physico-chemical properties of this compound are quite promising in terms of its characteristic drug like properties.

Conflict of Interests

The authors have not declared any conflict of interests.

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