HOSTED BY
ELSEVIER
Contents lists available at ScienceDirect
Journal of Acute Disease
journal homepage: www.jadweb.org

Document heading doi: 10.1016/S2221-6189(14)60078-0

Trypanocidal efficacy of two indigeneous ethanolic plant extracts (*Mimosa pigra* and *Ipomoea asarifolia*) against *Trypanosoma evansi* phospholipase A_2 activity

Yusuf Alkali[1], A. K. Gana[1], Abdulkadir A[2*], Nzelibe C. Humphrey[3]

[1]*national Cereal Research Institute Badeggi-Bida, Nigeria*

[2]*Federal University of Technology Minna, Nigeria*

[3]*Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria*

ARTICLE INFO

Article history:
Received 19 Dec 2014
Received in revised form 10 Jan 2015
Accepted 15 Jan 2015
Available online 26 Jan 2015

Keywords:
Phospholipase A_2
Indigeneous plant extract
Trypanosomiasis

ABSTRACT

Objective: To study the inhibitory activity of ethanolic extract from *Mimosa pigra* and *Ipomoea asarifolia* against *Trypanosoma evansi* (*T. evansi*) calcium dependent phospholipase A_2.
Methods: The calcium dependent phospholipase A_2 (E C 3.1.1.4) enzyme was isolated from *T. evansi* and purified to electrophoretic homogeneity under non denaturing conditions. It was solubilized from *T. evansi* cells recovered from white albino rats which were previously inoculated by intraperitoneal injection of infected camel blood. Two indigeneous ethanolic plant extracts used locally for treatment of trypanosomiasis were tested for the inhibition of phospholipases A_2.
Results: Double reciprocal plots of the initial velocity data of the inhibition by the indigenous plant extracts revealed a noncompetitive pattern of inhibition for the *Ipomoea asarifolia* and a competitive inhibition for *Mimosa pigra* in a dose dependent fashion. The extrapolated inhibition binding constant (Ki) of these extracts were found to be 2.0×10^2 μ g/mL and 1.12×10^2 μ g/mL respectively.
Conclusions: The low Ki values obtained for these extracts towards this enzyme are an indication of high affinity of the extract or the active components (present in the plants) are for these enzyme and therefore, could be explored to serve as a cheap source of *T. evansi* PLA2 antidote and as well help in designing a novel drug with high efficiency.

1. Introduction

Trypanosomiasis is a debilitating, severe and often fatal disease affecting both humans and animal is caused by a protozoan parasite called trypanosome. The disease is transmitted by bitting flies, which have acquired their infection from animals harbouring the pathogenic parasites[1-3]. The use of indigenous plant extract and conctions has been in practice for a very long time. It is as old as the world itself. *Mimosa pigra* and *Ipomoea asarifolia* are examples of such plants. Claims have been made that the roots are sniffed for head colds; a decoction of the leafy stem is used as a mouthwash for toothaches, and the fruits are used in eye medicines[1]. Apparently, it is also used for the treatment of snakebite in Africa[4]. In Sumatra, roasted and ground mimosa leaves are made into an infusion, which is drunk to treat a weak heart or weak pulse[5]. A decoction of dried and ground male and female parts are used in the same manner. In Mexico, an infusion of mimosa was traditionally used in Mayan medicine for treatment of diarrhoea. This knowledge led to leaves of mimosa and other legumes being phytochemically screened and tested for antimicrobial activity[6]. In phytochemical screening, flavonoides, quinones, saponins, sterols and tannins were detected in mimosa and *Ipomoea asarifolia*. Extracts of mimosa and *Ipomoea asarifolia* showed antimicrobial activity against

*Corresponding author: Abdulkadir Abdullahi, Federal University of technology Minna, Nigeria.
E-mail: abkad2008@yahoo.com

four bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans*, but not *Escherichia coli*. It was concluded that the use of mimosa, *Ipomoea asarifolia* and the other legumes tested could not be justified for treating diarrhoea.

However, the use of drugs for prevention and treatment of the trypanosomiasis has been in existence for many decades, but the speed by which the trypanosome develop resistance to each drug poses serious challenge to this approach in controlling the disease[7]. The use of chemoprophylactic drugs is expensive, time consuming and thus unsatisfactory to the long time problem of African animals' trypanosomiasis. Hence, a need for investigation into cost effective and more efficacious targets such as plant extracts that have been believed to have trypanocidal effect. Most part of Nigeria belong to the tsetse-fly infested belt especially the coastal areas bordering the two major rivers, Niger and Benue where the Nupe people are concentrated, constitute foci for trypanosomiasis. Over the years, these people have learnt from experience, the clinical signs of the disease and have developed method of treatment through the use of herbal medicine, but the efficacy has not been scientifically proven.

This work attempts to establish the inhibitory (trypanocidal) effect of *Ipomoea asarifolia* and *Mimosa pigra*a used among the Nupe ethnic group of Nigeria to treat animal and human trypanosomiasis. But the efficacy has not been scientifically proven owing to the current interest in the development of novel tryponocidal drug from plant product with zero side reactions. We found it imperative to initiate this work. In this piece, we communicate the tryponocidal activity of *Mimosa pigra* and *Ipomoea asarifolia* on *Trypanosoma evansi* (*T. evansi*).

2. Materials and methods

2.1. Infection of rat with T. evansi

The parasite *T. evansi* STIB 731-AA (IL-1392) was obtained from the Department of Veterinary Parasitology ABU Zaria. Parasites were passaged into a white albino rat (donor rat) by intraperitoneal inoculation. The levels of parasiteamia of the infected rats were monitored daily. The parasitaemia was monitored daily on a wet film preparation from tail vein blood. *T. evansi* was a gift from Dr. P. Audu of the Department of Biological Sciences Ahmadu Bello University, Nigeria , the parasite strain (*T. evansi*) STIB 731-AA (IL-1392).

Animal-fifteen mature albino rats free from infection were purchased from the Department of Pharmacology, Ahmadu Bello University, Nigeria.

The phospholipase activity of *T. evansi* was determined using the egg yolk coagulation method of Habermann and Neumann which was modified by Gomes and Pallabi[8]. Phospholipase activity was measured quantitatively (titration) by the initial rate of free fatty acid produced in micro moles per minute. The assay mixture contains 500 μL of lecithin, 50 μL of calcium chloride, and 100 μL of enzyme solution (in phosphate buffer 7.2) to give a total volume of 650 μL. The reaction was always initiated by the addition of the enzyme last. Thereafter, the enzyme was inactivated by heating at 100 ℃ for 1 min. The liberated fatty acid was titrated to an end point against 20 mm NaOH using phenolphthalein as indicator. The phospholipase A_2 activity was given as the number of moles of free fatty acid (average of 3 determination±SD) liberated by 1 mg of the enzyme[9].

2.2. Ion exchange chromatography

The ion exchange was carried out by method described by Nok *et al*[10]. All operations were maintained at about 4 degree centigrade. The crude enzyme was loaded on a buffer (pH 6.8) pre-equilibrated DEAE-cellulose column (2 cm×30 cm) and eluted by linear gradient between 0.02 and 0.3 M phosphate saline buffer (pH 6.8) forty fractions (40) of 5.0 mL each was collected and assayed for phospholipase activity and protein content. The dialysate was labeled as partially purified PLA_2.

2.3. Effect of indigenous plant extract on PLA_2 activity

Mimosa pigra and *Ipomea asarifolia* were two indigenous plants used traditionally for the treatment of trypanosomiasis. Ethanolic extracts of these plants were prepared in duplicate vig: 10 mg/mL (10%), 20 mg/mL (20%). Initial velocity studies were carried out. Results obtained from the initial velocity studies were used to determine K_M and Ki for the various extracts.

In all cases, the reaction was initiated by adding the enzyme last and the activity was stopped by boiling up to 90 ℃ and measured quantitatively by titration.

2.4. Effect of divalent cations on the activity of PLA_2

The enzyme was assayed for the effect of divalent cations at pH 7.2 and all other conditions remaining constant, except that, Ca^{2+}, the cofactor required for PLA_2 activity was replaced with copper, magnesium, and zinc.

2.5. Effect of anticoagulant

Substrate was prepared at concentrations between 2–10 mg/mL in triplicate. Each preparation was inoculated with 100 μL of PLA_2 and the activity assay as described previously. Inhibition analysis was made on similar preparations in the presence of 5×10^{-3} M of EDTA and haparin.

2.6. Effect of indigenous plant extract on PLA_2 activity

Mimosa pigra and *Ipomea asarifolia* were two indigenous plants used traditionally for the treatment of trypanosomiasis. Ethanolic extracts of these plants were prepared in duplicate vig: 10 mg/mL (10%), 20 mg/mL (20%). Initial velocity studies were carried out. Results obtained from the initial velocity studies were used to determine K_M and Ki for the various extracts.

3. Results

The inhibition mechanism of the antiicoagulant, heparin and EDTA shows noncompetitive pattern of inhibtion (Figure 1).

Double reciprocal plots of the initial velocity data of the inhibition by the extracts revealed a non competitive pattern of inhibition for *Ipomoea asarifolia* and a competitive inhibition for *Mimosa pigra* in a dose fashion PLA_2 (Figures 2 and 3). Extrapolated inhibition binding constant ki were found to be 50.0 μg/mL and 87.11 μg/mL for *Ipomoea asarifolia* and *Mimosa pigra* respectively (Figures 4 and 5).

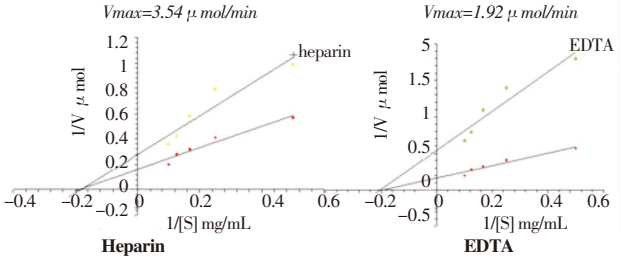

Figure 1. Effect of EDTA and heparin on PLA_2.

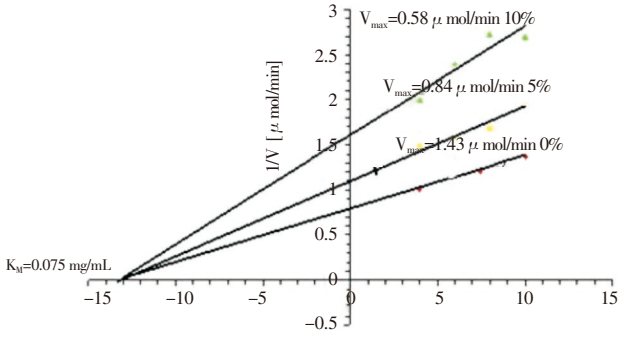

Figure 2. Effect of extract of *Ipomoea asarifolia* on PLA_2.

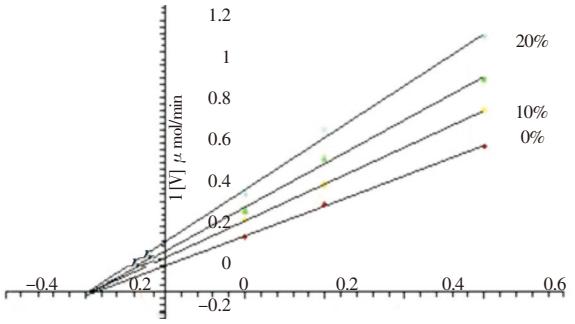

Figure 3. Effect of extract of *Mimosa pigra* on PLA_2.

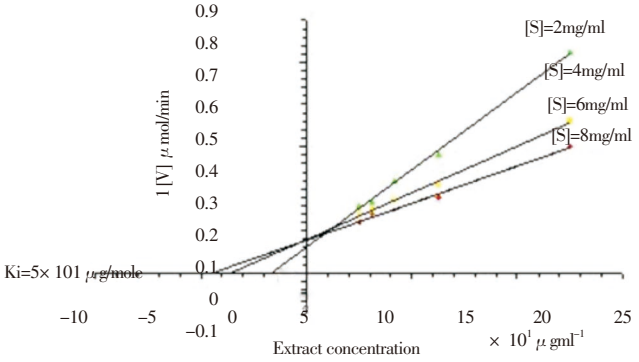

Figure 4. Determination of inhibition constant (Ki) of *Ipomea asarifolia* extract on PLA_2.

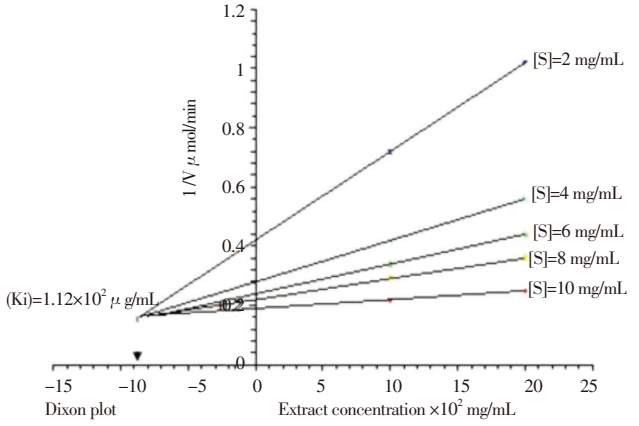

Figure 5. Determination of inhibition binding constant (Ki) of *Mimosa pigra* for PLA_2.

4. Discussion

PLA_2 has been isolated from various animals and plant source such as pancreas, snake and bee venoms and reported on platelet and phospholipase A_2[8,11,12]. The primary structure of human pancreatic PLA_2 has been reported[11]. A double reciprocal plot for the evaluation of inhibition type by *Ipomoea asarifolia* revealed non-competitive pattern with Ki values of 9.4 and 16.2 mg/mL, respectively. At these concentrations, the physiological index of efficiency of the enzyme (V_{max}/K_m) decreased from 22.4/h to 16.5 and 9.8/h respectively. The non-competitive inhibition pattern suggests that sites other than the active

could be involved in the inhibition. Since pre-incubation of the enzyme with *Ipomoea asarifolia* followed by dialysis failed to reverse the inhibition, these suggested that the *Ipomoea asarifolia*-PLA_2 interaction involved strong covalent forces affecting the structure.

The pattern of PLA_2 inhibition by the extract of *Mimosa pigra* shows that the active site of the enzyme is involved in the inhibitory action and that the extract is likely to contain some compounds or small molecules that serve as analogues of the PLA_2 substrate, by competing for the enzyme active site. Since the substrate (lecithin) and extract are competing for the same site on the enzyme, the K_M for the substrate shows an apparent increase in the presence of the extract. This can be seen in a double reciprocal plots as a shift in the X intercept ($-1/K_m$) and in the slope K_m/V_{max}). The effect of *Guiera senegalenses* against phospholipase A_2 revealed various inhibitions of the parasites by *Guiera senegalences* 10%, 20% and 30% which depicts a classical competitive inhibition which was dose dependent[9].

PLA_2 is a pathological enzyme found in several species of protozoa and snake venom[8]. It is known to be toxic to cell membrane, causing local cell and tissue damage as well as inflammatory effect[8,11,13]. The hydrolysis of the glycerophospholipids by $sPLA_2$ has great importance in the release of lipid mediators[14]. However, high phospholipase activity can lead to pathological implications. More recently, the phospholipase activity has been associated with angiogenesis, apoptosis and cancer. Arachidonate is metabolized into prostaglandins and to leukotrienes, potent mediators of immune suppression, cellular proliferation, tumor motility as well as invasion regulating tumor vascularization and metastasis in animal models. Beside enzymatic activity, $sPLA_2$ from snake venoms can be neurotoxic[15], myotoxic[13], cardiotoxic and can inhibit platelet aggregation and blood coagulation. Thus, PLA_2 induce various pharmacological effects including myotoxic and anticoagulant effect. The inhibition of PLA_2 can therefore be significant in ameliorating the cell damaging effect by the PLA_2 on red blood cells which lead to anaemia[10]. The low Ki values obtained for these extracts towards this enzymes are an indication of high affinity the extract or the active components (present in the plants) have for these enzyme and therefore an indication that it could be explored to serve as a good source of *T. evansi* PLA_2 antidote and as well help in designing a novel drug, with high efficiency.

Conflict of interest statement

The authors report no conflict of interest.

References

[1] Holland WG, My LN, Dung TV, Thanh NG, Tam PT, Vercruysse J, et al. The influence of *T. evansi* infection on the immuno-responsiveness of experimentally infected water buffaloes. *Vet Parasitol* 2001; **102**: 225-234.

[2] Chiou SH, Raynor RL, Zheng B, Chambers TC, Kuo JF. Cobra venom cardiotoxin (cytotoxin) isoforms and neurotoxin: comparative potency of protein kinase C inhibition and cancer cell cytotoxicity and modes of enzyme inhibition. *Biochemistry* 1993; **32**: 2062-2067.

[3] Habila N, Humphrey NC, Abel AS. Trypanocidal potentials of *Azadirachta indica* seeds against *Trypanosoma evansi*. *Vet Parasitol* 2011; **180**: 173-178.

[4] Irvine FR. *Mody plants of Ghana with special reference to their uses*. London: Oxford University press; 1961.

[5] Grosvenor PW, Gothardd PK, McWilliams NC, Supriomo A, Gray DO. Madicinal plants from Rian Province Sumatra, Indonesia. Part1: uses. *J Ethnophamacol* 1995; **45**(2): 75-95.

[6] Rosodo-Vallado M, Brito-Loeza W, Mena-Rejón GJ, Quintero-Marmol E, Flores-Gimdo JS. Antimicrobial activity of Fobaceae species used in Yucatan traditional medicine. *Fitoterapia* 2000; **71**: 570-573.

[7] Losos JG. *Animal trypanosomiasis in infectious diseases of domestic anaimals*. Canada: Int'L Development Research Centre; 1986.

[8] Gommes A, De P. Hannahpep: a novel fibrinolytic peptide from the India Cobra (*Ophiophagus hanna*) venom. *Biochem Biophys Res Commun* 1999; **266**: 488-491.

[9] Sallau AB, Njoku GC, Olabisi AR, Wurochekke AU, Abdulkadir AA, Isah S, et al. Effect of *Guiera senegalensis* leaf extract on some *Echis carinatus* venom enzymes. *J Med Sci* 2005; **5**: 280-283.

[10] Nok AJ, Esievo KA, Ibrahim S, Ukoha AI, Ikediobi CO. Phospholipase A_2 from Trypanosoma congolense: characterization and hematological properties. *Cell Biochem Funct* 1993; **11**: 125-130.

[11] Forst S, Weiss J, Elsbach P, Maraaganore J, Reardon I, Heinrikson R. Structural and functional properties of a phospholipase A2 purified from an inflammatory exudate. *Biochemistry* 1986; **25**: 8381-8385.

[12] Horigome K, Hayakawa M, Inoue K, Nojima S. Purification and characterization of phospholipase A_2 released from rat platelets. *J Biochem* 1987; **101**: 625-631.

[13] Omer OH, Mousa HM, Al-Wabel N. Study on the antioxidant on pig performance and vaccination against classical swine fever. *Vet Parasitol* 2007; **145**: 142-145.

[14] Dennis EA. Phospholipase A2 mechanism inhibition and role in arachidonic acid release. *Drug Dev Res* 1987; **10**: 205-220.

[15] Prijatelj P, Charnay M, Ivanovski G, Jenko Z, Pungerčar J, Križajc I, et al. The C-terminal and beta-wing regions of ammodytoxin A, a neurotoxic phospholipase A_2 from Vipera ammodytes ammodytes, are critical for binding to factor Xa and for anticoagulant effect. *Biochimie* 2006; **88**(1): 69-76.