



DNA Binding Interactions and DNA Topoisomerase I Inhibition Activities of Crude Extracts from *Annona Squamosa* (L.) and *Annona Muricata* (L.) Fruits

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Abstract: Some plants metabolites serve as antiprotozoal and antitumour by binding to nuclear enzyme; DNA Topo I affecting DNA function and cell survival. This study was aimed at screening DNA binding interactions and DNA Topo I inhibitory activity of crude extracts from fruits of *Annona muricata* (L) and *Annona squamosa* (L) which can form the basis of developing efficacious, safe and low cost antiprotozoal and antitumor agents. Aqueous, Methanolic, Ethyl acetate and Hexane extracts from fruits of two hypothesized antiprotozoal and antitumour plants; *Annona muricata* (L) and *Annona squamosa* (L) were screened for DNA-binding interaction and DNA Topo I inhibition. For DNA-methyl green test, 50 μ L crude extracts were incubated with 200 μ L DNA-methyl green in darkness at 25°C for 24 hours. Absorbance decrease at 650 nm using UV-vis spectrophotometer was calculated as a percentage of untreated DNA-methyl green value whereas with IC₅₀ calculated by regression analysis. For DNA Topo I inhibitory activity, crude extracts were incubated in 10 μ g/mL with 0.5 μ g of supercoiled pBR322 DNA and 1U of DNA Topo I at 37°C for 2 hours, reaction terminated using stop buffer containing 3% SDS, 60 mM EDTA, 50% glycerol, 0.25% bromophenol blue. Products were determined by electrophoresis on 1% agarose gel in Tris-acetate-EDTA (TAE) running buffer at 65 V/cm for 2 hours. 24 extracts were studied, percentage decrease in absorbance were between 18.14 \pm 2.67 - 38.06 \pm 1.47 (Aqueous), 17.14 \pm 2.67 - 41.01 \pm 1.09% (Methanolic), 9.05 \pm 1.67 - 20.50 \pm 2.01% (Ethyl acetate) and 4.04 \pm 1.12 - 10.09 \pm 1.39% (Hexane)., IC₅₀ values were between 50 μ g/mL - 100 μ g/mL (6), 100 μ g/mL - 150 μ g/mL (8), 150 μ g/mL - 200 μ g/mL (7) and <200 μ g/mL (3). The activity against DNA Topo I mediated relaxation of supercoiled pBR322 DNA at 5 μ M, 25 μ M and 100 μ M observed in 8 hits with percentage decrease in absorbance between 17.14 \pm 2.67 - 40.01 \pm 1.09% with IC₅₀ between 62.97 \pm 3.37 μ g/mL - 131.37 \pm 10.77 μ g/mL. The extracts of *A. muricata* and *A. squamosa* showed DNA Topo I inhibitory activities by inhibiting the relaxation of supercoiled DNA pBR322. However, further studies need to be conducted on the purified fractions of aqueous and methanolic extracts.

Keywords: Topoisomerase I Inhibitors, DNA-Binding, *Annona squamosa*, *Annona muricata*

1. Introduction

Protein and DNA-binding interaction is important in development of both anti-tumor and antiprotozoal drugs such as antileishmanial agents [1, 2]. Studies have investigated the inhibitory effect of medicinal plant extracts on protein and DNA sequences [3-12, 2]. However, limitations in solubility

and low bioavailability necessitate high doses for effectiveness of most chemotherapeutic agents [13]. Therefore, combination therapy of natural products alongside antibiotics or synthetic drugs is an emerging trend in leishmaniasis therapy [14-16]. Based on the mechanism of action of leishmanicidal such as inhibition of specific enzymes like DNA Topo I and DNA Topo II [1, 2, 17-19], the

genes coding for these targets can be targeted for development of newer, cheap, safe and efficacious antileishmanials. Although topoisomerase blockers from plant secondary metabolites have been identified [1, 2, 20], the search for more compounds with topoisomerase blocking activity focusing on antiprotozoal is paramount. In the past few decades, combined therapy involving antioxidant molecules from plants have come into focus in treatment of degenerative diseases [21-25] and antiprotozoal diseases [14, 15, 26-28]. The Annonaceae fruits are cultivated in several parts of Kenya and have been traditionally used for the treatment of different illnesses including antivenin [29-32]. Although, several studies have concentrated on antioxidant, chemopreventive and hepatoprotective properties of *A. squamosa* and *A. muricata* extracts [33-39], the Kenyan varieties of the same plant species have been neglected [25, 28, 32]. Furthermore, the above cited studies concentrated on anti-cancer and antiprotozoal properties of the roots, leaves, seeds and bark of the plants in question [40-46]. This study reports on the fruits of *Annona muricata* (L) and *Annona squamosa* (L) as excellent source for DNA binding and DNA Topo I inhibitory activity, which can form the basis of developing efficacious, safe and low cost antiprotozoal and antitumor agents.

2. Materials and Methods

2.1. Collection of Plant Materials

The ripe fresh fruits of *A. muricata* and *A. squamosa* were collected in the period between March and September 2014 from farms in coast province (Kilifi and Kwale Counties) of Kenya. The National Museum of Kenya, Nairobi, identified the species where voucher numbers for *A. muricata* and *A. squamosa* were deposited. The harvested fruits were washed with chlorinated water to retard aging and removal of fungi and bacteria. The pulp, peel and seeds from the fruits were separated from each other and then dried using a constant temperature and humidity chamber (Tokyo Thermo Tech Co. Ltd, Japan) set at 40°C and 95% relative humidity. Dry pulp, peel and seeds were then separately grounded into fine powder using a grinding machine (Mitamura Riken, Kogyo Inc. Tokyo, Japan). The parts of the fruits collected were grounded separately into powder, weighed using a top-loading balance, transferred into polythene bags, sealed, and stored at 4°C until extraction.

2.2. Extraction of Phytochemicals

Aqueous extraction was carried out where 50 g of each of the dried pulp, peel and seeds from *A. muricata* and *A. squamosa* fruits was macerated in 100 mL sterile distilled water in a Warring blender for 10 minutes. The macerate was first filtered through double-layered muslin cloth and then centrifuged at 4000 g for 30 minutes. Thereafter, the supernatant was filtered through Whatman No. 1 filter paper. The extracts described and abbreviated in Table 2 were finally preserved aseptically in sterile airtight bottle at 4°C for later use [47]. The organic solvents extraction was carried

out using 50 g of powdered pulp, peel and seeds from *A. muricata* and *A. squamosa* fruits sequentially using 100 mL of solvents of increasing polarity, starting with n-hexane followed by ethyl acetate (EA) and finally Methanol (MeOH) for 48 hours each with occasional swirling to ensure thorough extraction. The extracts were decanted and filtered through Whatman filter paper and the macerate steeped in solvents (n-hexane, ethyl acetate and MeOH) again for 48 hrs. Extraction process was repeated twice and the filtrates combined and concentrated on a rotary vacuum evaporator (Bibby Sterilin Ltd, RE 100B, UK) under reduced pressure at a temperature of 50°C and packed and stored in airtight bottles at 4°C for later use (Biba *et al.*, 2013) as described and abbreviated in Table 1.

Table 1. Abbreviations of different crude extracts of *A. muricata* and *A. squamosa* pulp, peel and seeds.

Extract Abbreviation	Full name of the extract
ASPUAE	<i>Annona squamosa</i> pulp aqueous extract
ASPUME	<i>Annona squamosa</i> pulp methanol extract
ASPUEAE	<i>Annona squamosa</i> pulp ethyl acetate extract
ASPUHE	<i>Annona squamosa</i> pulp hexane extract
ASPEAE	<i>Annona squamosa</i> peel aqueous extract
ASPEME	<i>Annona squamosa</i> peel methanol extract
ASPEEAE	<i>Annona squamosa</i> peel ethyl acetate extract
ASPEHE	<i>Annona squamosa</i> peel hexane extract
ASSAE	<i>Annona squamosa</i> seeds aqueous extract
ASSME	<i>Annona squamosa</i> seeds methanol extract
ASSEAE	<i>Annona squamosa</i> seeds ethyl acetate extract
ASSHE	<i>Annona squamosa</i> seeds hexane extract
AMPUAE	<i>Annona muricata</i> pulp aqueous extract
AMPUME	<i>Annona muricata</i> pulp methanol extract
AMPUEAE	<i>Annona muricata</i> pulp ethyl acetate extract
AMPUHE	<i>Annona muricata</i> pulp hexane extract
AMPEAE	<i>Annona muricata</i> peel aqueous extract
AMPEME	<i>Annona muricata</i> peel methanol extract
AMPEEAE	<i>Annona muricata</i> peel ethyl acetate extract
AMPEHE	<i>Annona muricata</i> peel hexane extract
AMSAE	<i>Annona muricata</i> seeds aqueous extract
AMSME	<i>Annona muricata</i> seeds methanol extract
AMSEAE	<i>Annona muricata</i> seeds ethyl acetate extract
AMSHHE	<i>Annona muricata</i> seeds hexane extract

2.3. DNA-binding Interactions Assays

The DNA-methyl green test was performed as described by Attard and Pacioni [3]. Fifty (50) µL of the extracts were incubated with 200 µL of DNA-methyl green in the dark at 25°C for 24 hours. The decrease in absorbance at 650 nm using a UV-vis spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan) was calculated as a percentage of the untreated DNA-methyl green absorbance value. The median inhibitory concentration (IC₅₀) was calculated by regression analysis. Cucurbitacin E and Dexamethasone were used as potent and moderate positive controls, respectively.

2.4. Topoisomerase I Inhibitory Activity Assays

The Calf thymus DNA Topo I was purchased from Sigma and assayed using TopoGen (Columbus, OH, U.S.A.) assay kit as described by Pastor and Cortes [48] with some

modifications. Different concentrations (1, 5, 10, 25, 50 and 100 μM) of tested extracts were prepared using DMSO. Positive control, camptothecin (CPT), was prepared at the concentration of 10 μM . The DNA Topo I inhibitory activity was measured by assessing the relaxation of supercoiled pBR322 plasmid DNA. The reaction mixture (20 μL each), containing 35 mM Tris-HCl (pH 8.0), 72 mM KCl, 5 mM MgCl_2 , 5 mM dithiothreitol (DTT), 5 mM spermidine, 0.01% bovine serum albumin (BSA), 0.5 μg pBR322 plasmid DNA, 1.0 U calf thymus DNA Topo I, and 0.2 μL various concentrations of tested extracts, were incubated at 37°C for 30 min. The reactions were terminated by adding dye solution containing 1% SDS, 0.02% bromophenol blue, and 50% glycerol. The mixtures were applied to 1% agarose gel and subjected to electrophoresis for 1 h in Tris-borate-EDTA buffer (0.089 mM). The gel was stained with Gelred and visualized under UV illumination, photographed with a Gel imaging system.

2.5. Data Analysis

The numerical data (percentage decrease in absorbance and IC_{50}) were analyzed using mean separation by GenStat discovery 14th Edition [49] through Fischer least significance difference. Comparisons of multiple means was done by Analysis of variance (ANOVA) followed by Duncan's test, $P < 0.05$ considered statistically significant. The results were expressed as mean \pm standard error of the mean.

3. Results

3.1. DNA Binding Activity

The crude plant extracts are complex matrices containing several phytochemicals. Therefore, the IC_{50} values of the studied extracts were expected to be higher than those for pure compounds. The IC_{50} values and the percentage decrease in absorbance obtained for the DNA-methyl green assay as an indicator of plant extract (s) DNA binding interaction are presented in Table 2.

The extracts with higher IC_{50} values had low percentage inhibition than the extracts with low IC_{50} values (Table 2). The recorded percentage decrease in absorbance of the aqueous extracts (ASPUAE, ASPEAE, ASSAE, AMPUAE, AMPEAE and AMSAE) ranged between 18.14 \pm 2.67% (AMPUAE) and 38.06 \pm 1.47 (ASPEAE) with IC_{50} values of ranges between 67.66 \pm 2.44 $\mu\text{g/mL}$ (ASPEAE) and 137.44 \pm 33.33 $\mu\text{g/mL}$ (AMPUAE). The methanolic extracts (ASPUME, ASPEME, ASSME, AMPUME, AMPEME and AMSME) followed with percentage decrease in absorbance ranges between 17.14 \pm 2.67% (AMSME) and 41.01 \pm 1.09% (AMPEME), IC_{50} values of 62.97 \pm 3.37 $\mu\text{g/mL}$ (AMPEME) and 159.79 \pm 9.44 $\mu\text{g/mL}$ (AMPUME). The ethyl acetate extracts (ASPUEAE, ASPEEAE, ASSEAE, AMPUEAE, AMPEEAE and AMSEAE) percentage decrease in absorbance ranged between 9.05 \pm 1.67% (AMSEAE) and 20.50 \pm 2.01% (AMPEEAE) with IC_{50} values of 115.87 \pm 7.67 $\mu\text{g/mL}$ (AMPEEAE) and 171.99 \pm 19.47 $\mu\text{g/mL}$ (AMSEAE). The hexane extracts (ASPUHE, ASPEHE,

ASSHE, AMPUHE, AMPEHE and AMSHE) had the lowest affinity to bind DNA with binding percentages decrease in absorbance ranging between 4.04 \pm 1.12% (AMSHE) and 10.09 \pm 1.39% (AMPUHE) with IC_{50} values of 165.97 \pm 24.65 $\mu\text{g/mL}$ (AMPUHE) and 270.98 \pm 37.57 $\mu\text{g/mL}$ (AMSHE). Out of the 24 extracts studied, none of them had IC_{50} values 0 – 50 $\mu\text{g/mL}$ while 6 extracts had IC_{50} values between 50 $\mu\text{g/mL}$ – 100 $\mu\text{g/mL}$. The IC_{50} ranging between 100 $\mu\text{g/mL}$ – 150 $\mu\text{g/mL}$ had 8 extracts, the range of 150 $\mu\text{g/mL}$ – 200 $\mu\text{g/mL}$ had 7 extracts while only 3 extracts had IC_{50} values <200 $\mu\text{g/mL}$.

Table 2. Percentage decrease in absorbance of DNA methylene green and IC_{50} values of *A. muricata* and *A. squamosa* pulp, peel and seeds aqueous, methanol, ethyl acetate and hexane extracts.

Extract	% Decrease in Absorbance	IC_{50} ($\mu\text{g/mL}$)
ASPUAE	20.50 \pm 2.01	108.42 \pm 7.67
ASPUME	18.14 \pm 1.12	133.66 \pm 11.45
ASPUEAE	11.14 \pm 1.67	156.17 \pm 5.67
ASPUHE	7.05 \pm 1.98	223.20 \pm 32.89
ASPEAE	38.06 \pm 1.47	67.66 \pm 2.44
ASPEME	22.65 \pm 2.28	104.45 \pm 4.67
ASPEEAE	12.14 \pm 1.67	158.08 \pm 15.55
ASPEHE	6.77 \pm 1.11	227.59 \pm 41.87
ASSAE	26.46 \pm 1.37	95.69 \pm 9.01
ASSME	36.76 \pm 1.47	66.80 \pm 6.07
ASSEAE	16.14 \pm 3.01	147.54 \pm 13.09
ASSHE	8.84 \pm 1.27	177.20 \pm 21.97
AMPUAE	18.14 \pm 2.67	137.44 \pm 33.33
AMPUME	11.74 \pm 2.67	159.79 \pm 9.44
AMPUEAE	19.94 \pm 2.00	130.59 \pm 6.44
AMPUHE	10.09 \pm 1.39	165.97 \pm 24.65
AMPEAE	26.76 \pm 1.47	80.99 \pm 4.48
AMPEME	41.01 \pm 1.09	62.97 \pm 3.37
AMPEEAE	20.50 \pm 2.01	115.87 \pm 7.67
AMPEHE	8.01 \pm 2.19	189.54 \pm 28.65
AMSAE	30.67 \pm 1.47	77.46 \pm 5.67
AMSME	17.14 \pm 2.67	131.73 \pm 10.77
AMSEAE	9.05 \pm 1.67	171.99 \pm 19.47
AMSHE	4.04 \pm 1.12	270.98 \pm 37.57
Positive (Cucurbitacin)	87.67 \pm 3.45	20.19 \pm 1.12
Negative (Dexamethasone)	2.33 \pm 0.98	33.76 \pm 2.02
Mean values \pm SEM, (n=3)		

3.2. DNA Topoisomerase I Inhibitory Activity

Eight (8) of the 24 extracts with percentage inhibition ranging between 17.14 \pm 2.67% (AMSME) and 40.01 \pm 1.09% (AMPEME) with IC_{50} values ranging between 62.97 \pm 3.37 (AMSME) and 131.37 \pm 10.77 (AMSME) were investigated for their DNA Topo I inhibitory activities. Screening of these eight extracts (ASPEAE, ASPEME, ASSAE, ASSME, AMPEAE, AMPEME, AMPEEAE and AMSAE) indicated activity against Topo I mediated relaxation of supercoiled DNA at the concentration of 100 μM (Figure 1).

The Topo I inhibition activity of the eight (8) hits were further tested at lower concentrations of 5 μM and 25 μM . At a concentration of 25 μM , five extracts in lane 3, lane 4, lane 5, lane 6 and lane 8 showed DNA Top I inhibitory activities (Figure 2). Negative Topo I inhibitory activities were observed in lane 1, 2 and 7.

At a concentration of 5 μM three AMPEME (lane 3), ASSME (lane 5) and ASPEAE (lane 8) exhibited DNA Topo

I inhibitory activity while negative DNA Topo I inhibitory activity was observed in five extracts; AMSAE lane 1, AMPEEA lane 2, AMPEAE lane 4, ASSAE lane 6 and ASPEME lane 7 (Figure 3).

	1	2	3	4	5	6	7	8	9	10	11
Relaxed DNA											
Supercoiled DNA											
pBR322 DNA (0.5 µg)	+	+	+	+	+	+	+	+	+	+	+
DNA TOPI (1 U)	+	+	+	+	+	+	+	+	+	+	-
CPT (10 µM)	-	-	-	-	-	-	-	-	+	-	-

Figure 1. DNA Topo I inhibitory activities of Extracts at 100 µM. Extracts (DNA + Topo I + tested compounds); ASPEAE, ASPEME, ASSAE, ASSME, AMPEAE, AMPEME, AMPEEA and AMSAE in lane 1-8 respectively. Positive control (DNA + Topo I + CPT) lane 9. Negative control (DNA + Topo I) lane 10 and DNA alone lane 11.

	1	2	3	4	5	6	7	8	9	10	11
Relaxed DNA											
Supercoiled DNA											
pBR322 DNA (0.5 µg)	-	-	+	+	+	+	-	+	+	+	+
DNA TOPI (1 U)	+	+	+	+	+	+	+	+	+	+	-
CPT (10 µM)	-	-	-	-	-	-	-	-	+	-	-

Figure 2. DNA Topo I inhibitory activities of Extracts at 25 µM (DNA + Topo I + tested compounds). Positive extracts; AMSAE (lane 1), AMPEME (lane 3), AMPEAE (lane 4), ASSME (lane 5), ASSAE (lane 6) and ASPEAE (lane 8). Negative extracts; AMPEEA (lane 2) and ASPEME (lane 7). Positive control; (DNA + Topo I + CPT) (lane 9, Negative control (DNA + Topo I) (lane 10) and DNA alone (lane 11).

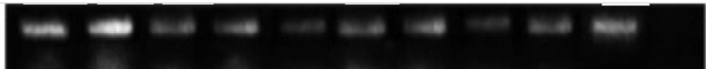

	1	2	3	4	5	6	7	8	9	10	11
Relaxed DNA											
Supercoiled DNA											
pBR322 DNA (0.5 µg)	-	-	+	-	+	-	-	+	+	+	+
DNA TOPI (1 U)	+	+	+	+	+	+	+	+	+	+	-
CPT (10 µM)	-	-	-	-	-	-	-	-	+	-	-

Figure 3. DNA Topo I inhibitory activities of Extracts at 5 µM (DNA + Topo I + tested compounds). Positive extracts; AMPEME (lane 3), ASSME (lane 5) and ASPEAE (lane 8). Negative extracts; AMSAE (lane 1), AMPEEA (lane 2), AMPEAE (lane 4), ASSAE (lane 6) and ASPEME (lane 7). Positive control; (DNA + Topo I + CPT) (lane 9). Negative control (DNA + Topo I) (lane 10) and DNA alone (lane 11).

4. Discussion

The popularity, potential and use of *A. squamosa* and *A. muricata* in treatment of various diseases such as malaria [50], Diabetes [51], leishmaniasis [46, 52-55], trypanosomiasis [45, 56], cancer [40, 41, 57-59] among others is increasing due to ethnobotanical report on its selective cytotoxic activity [32, 40, 41, 60]. *In vitro* studies have shown more selective toxicity to cancer cell lines than to normal cells [32, 37, 40, 41]. The DNA methyl green bioassay is one of the simplest and comprehensive techniques used to study DNA binding interactions with other compounds with a high throughput [3]. During the assay, the methyl green was found to bind quantitatively to DNA forming a DNA-methyl green complex [3]. The affinity determines the displacement of methyl green, leading to a colourless carbinol [61-63]. In this study, DNA methyl green assay was used to determine the affinity of *A. squamosa* and *A. muricata* fruits pulp, peel and seeds aqueous, methanol, ethyl acetate and hexane extracts.

Studies have shown that pure compounds of plant origin may be associated with low IC_{50} values [64, 65]. In other words, due to the fact that the crude extracts of *A. muricata* and *A. squamosa* fruit peel, pulp and seeds being complex matrices with several phytochemicals [32, 66], the IC_{50} values are expected to be higher than those of pure compounds [3]. Therefore, it is reasonable that in the case of extracts investigated in this study, higher IC_{50} values were expected. The studies involving DNA binding interaction of different plant species extracts with IC_{50} values $>70 \mu\text{g/mL}$ may be considered as active [3]. Therefore, only 3 extracts out of the 24 extracts involved in this study (AMPEME, ASPEAE and ASSME) translating to 12.5% had IC_{50} values $<70 \mu\text{g/mL}$ thus had the ability to displace methyl green from the methyl green DNA complex. It is likely that these extracts contain compounds that may act as intercalating agents at the DNA level. All the active extracts (AMPEME, ASPEAE and ASSME) were extracted using water and methanol, which are polar solvents.

A relationship between the affinity of the extracts to bind DNA and phytochemicals present has been reported [3, 32]. In this study, alkaloids were not the predominant phytochemical in AMPUAE (IC_{50} ; $137.44 \pm 33.33 \mu\text{g/mL}$) and ASPUAE (IC_{50} ; $108.42 \pm 7.67 \mu\text{g/mL}$) as reported by Chimbevo [32] and had low affinity to interact with the DNA in the methyl green complex. Studies indicates that active polar aqueous extract (ASPEAE; IC_{50} , 67.66 ± 2.44), polar methanolic extracts (AMPEME; IC_{50} ; $62.97 \pm 3.37 \mu\text{g/mL}$ and ASSME; IC_{50} ; $66.80 \pm 6.09 \mu\text{g/mL}$) contained abundant terpenoids, flavonoids, phenols and saponins [32, 66]. In other studies, a correlation between the phytochemical class and DNA-methyl green displacement activity and ability to inhibit DNA topoisomerase II was observed [4]. The percentage decrease in absorbance of DNA methyl green in these studies were even superior compared to studies conducted by Attard & Pacioni [3] on other plant species.

Majority of the extracts with DNA binding interaction in the study were from polar solvents which confirms studies by Attard and Pacioni [3] and Correa and co-workers [9] on other plant species. It has been reported that naturally occurring products intercalating with DNA to be alkaloids [6-7, 67-68], while others do not intercalate with DNA [5, 69]. Variation in [DNA]/[Extract] molar ratio [70], changes in ionic strength [5], size and structure of the ligands may affect the interaction [71] are among the factors that may affect ability of the extracts to interact with the DNA. Thus, some of these factors may be responsible for the low affinity DNA interaction found in this study.

Since 37.5% of the extracts studied showed a DNA interaction with decrease in percentage absorbance of DNA methyl green $>20.00\%$, it can thus be inferred that the *A. muricata* and *A. squamosa* fruits pulp, peel and seeds aqueous, methanol, ethyl acetate and hexane extracts have the potential as a source of phyto-compounds with possible pharmacological applications. Studies have been conducted to investigate the effects of reducing the molecular weight of antileishmanial compounds on DNA binding affinity, antileishmanial and cytotoxicity activity [72]. Although the DNA used was not isolated from the leishmania parasite, the study provides insights on the potential of the extracts used to interact with DNA from the parasite. It is important to mention that few literature reports exist on the evaluation of ethnopharmacological bioactivity of these *A. muricata* and *A. squamosa* fruits pulp, peel and seeds extracts through DNA binding interaction. It is therefore necessary to continue with the isolation, identification, and evaluation of the secondary metabolites responsible for this DNA interaction among the more active extracts obtained from this study. This provides base line data for *A. muricata* and *A. squamosa* fruits pulp, peel and seeds extracts to develop antileishmanial agents.

Most of the topoisomerase inhibitors in use are sourced from natural products [2] and thus there is considerable interest in identifying novel inhibitors from plant products. In continuation of the search for new topoisomerase inhibitors, the study investigated aqueous, methanol, ethyl acetate and hexane extracts of *A. squamosa* and *A. muricata* from pulp, peel and seeds. Although studies have demonstrated potential DNA binding interactions of plant extracts [11, 12, 31, 73-75], their effect on DNA topoisomerases was not explored previously. The observed inhibition of DNA Topo I at very low concentration ($0.75 \mu\text{M}$) in this study with mixtures of different plant extracts [76], suggests synergistic association between compounds present in the different extracts in inhibition of DNA Topo I [2]. Adverse toxic side effects limit the evaluation and use of DNA Topo I inhibitors for the treatment of various illnesses including form of cancers and leishmaniasis [72, 76].

However, isolation of pure compounds from the crude extracts may increase, enhance or improve the DNA Topo I inhibitory activities [2]. Thus, the need for natural non-toxic Topo I inhibitors, which may have possible cancer and leishmanial chemotherapeutic potentials. The study

demonstrates inhibitory effect of *A. muricata* and *A. squamosa* fruits pulp, peel and seeds extracts on synthetic DNA Topo I at 5 μ M. However, the effect of mixture of different extracts and concentration on DNA Topo I inhibition was not investigated. Studies carried on DNA Topo I inhibitory activity revealed that majority of the active extracts are from polar solvents and contain flavonoids [2]. Although synthetic DNA Topo I was used, the study demonstrate that plant extracts are potent inhibitors of DNA Topo I, a property that may be helpful in development of anticancer and antileishmanicidal agents. Thus *in vitro* and *in vivo* studies of *A. muricata* and *A. squamosa* fruits pulp, peel and seeds extracts using purified DNA and DNA Topo I from the parasite and host should be conducted.

5. Conclusions

DNA binding and DNA Topo I inhibitory activities, have value in rational drug design of efficacious, safe and low cost anticancer and antileishmanial agents. Overall, the results of this study contribute to the chemotaxonomic understanding of the family Annonaceae, especially the genus *Annona* growing in Kenya. Besides, the results obtained confirms the importance of the selection of plant extracts used in folk medicine in screening programs in the search for new anticancer and antileishmanial agents. The knowledge and information from this study highlight the potential usefulness of *A. muricata* and *A. squamosa* fruits, which is paramount as it adds urgency to the search for new cancer and leishmaniasis fighting strategies such as good protein energy (PE) or Protein calorie (PC) as supportive therapy for the diseases.

6. Recommendations

The study was an attempt to determine the DNA-Protein Binding interaction and DNA Topo I inhibitory activities of *Annona muricata* (L.) and *Annona squamosa* (L.) fruits extracts growing in the coast region of Kenya. Investigation on the crude extracts from *A. muricata* and *A. squamosa* fruits to focus on isolation and characterization of the constituents present with the aim of understanding mechanisms of action, and encourage continued *in vitro* and *in vivo* investigations. Besides, metabolic studies are also necessary to determine whether digestive processes decrease or increase activity of the active compounds is recommended.

Author's Contribution

This work was carried out in collaboration between all the authors. 'Author LMC' designed the study developed the protocol and performed the experimental analysis of the study. 'Authors LMC, SE, FMM and GKG' managed the literature searches, statistical analysis and development of the manuscript. All authors read and approved the final draft of the manuscript.

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