

In Vitro Antiproliferative Activity of Aqueous Root Bark Extract of *Cassia abbreviata* (Holmes) Brenan

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Abstract

Cancer is referred to as uncontrolled abnormal proliferation of body cells. Currently cancer treatment and management is a challenge due to complexity of the disease, toxicity of chemotherapy, unaffordability of treatment and severe side effects. Therefore, it is imperative to investigate complementary and alternative medicine for leads and development of anticancer drugs. *Cassia abbreviata* (*C. abbreviata*) has been traditionally used for its ethnotherapeutic properties and pharmacological activities. It has been reported to possess antiproliferative activity, though there is no scientific evidence to validate this claim. The main objective of this study was to determine the antiproliferative activity of aqueous root bark extract of *C. abbreviata* on hepatocellular carcinoma (HCC), vero, and macrophage cell lines *in vitro*. The antiproliferative activity of aqueous root bark extract of *C. abbreviata* was determined using MTT assay. The results showed that aqueous root bark extract of *C. abbreviata* has antiproliferative activity against HCC, vero and macrophage cell lines. The extract had the highest antiproliferative activity against HCC cell line with an IC₅₀ of 1.49 µg/ml as compared to 81.08 µg/ml and 128.38 µg/ml in macrophages and vero cells respectively. The IC₅₀ observed on non-cancerous normal cells (macrophages and vero cells) indicated that the plant extract had little antiproliferative effects on normal cells hence regarded as safe. The extract contained flavonoids, phenols, tannins and saponins. In conclusion the antiproliferative activity of aqueous root bark extract of *Cassia abbreviata* observed could be attributed to the phytochemicals present in this plant extract. The results of this study, validates the claim that aqueous root bark extract of *C. abbreviata* has antiproliferative activity and justifies its use in herbal medicine.

Keywords: Cancer; Antiproliferation; Macrophage cells; Vero cells; IC₅₀

Introduction

Cancer is a leading cause of death in developed world and second cause of mortality in developing world. This is blamed on the rise in cancer-related lifestyles including smoking, lack of physical exercise, junk foods, environmental threats, costly medication and treatment. Cancer refers to a group of diseases that can affect any part of the body. It is a rapid growth of abnormal cells that grow beyond their normal cell division. It is also defined as manifestation of malfunctions of the immune system, as malignant cells escape recognition and elimination [1-4].

Cancer pathology is featured by rapid and uncontrolled cell proliferation that forms tumor cells, metastasize throughout the body and initiate abnormal growth of neoplastic tissue on other sites in the body. Conventional anticancer drugs have pharmacological effects of anti-proliferation, anti-inflammation, cytotoxicity, induction of differentiation, cell-cycle arrest and induction of apoptosis and/or autophagy, anti-invasion and anti-metastasis. However, they are characterized by challenges of drug resistance, drug toxicity, severe side effects and unaffordability [5,6].

Herbal medicine is an important segment of human health care in developing world, particularly Africa. The dependence of Africa on herbal medicine has been due to affordability, accessibility, local availability and acceptance by the local society. Furthermore, the available modern facilities of medicine such as hospitals, equipments and medical personnel are limited and not available to local communities making herbal medicine a golden alternative. Effectiveness of some herbal treatments to conditions such as malaria is well documented [7,8]. Therefore, it is important to evaluate alternative sources of anticancer drugs that are affordable with less drug toxicity.

Drugs from herbal sources are defined as affordable and safe for consumption. The number of medicinal plants used in traditional medicine is large but only few have been investigated for their pharmacological activity. Since ancient times, humans have used traditional medicines for human health care with terrestrial plants occupying a significant therapeutic role [9,10].

Cassia abbreviata is a leguminous tree that belongs to Caesalpinioideae family. It has light brown bark, rounded crown. The leaves are yellowish compound, with 5-12 pairs, and the pods which are black and cylindrical in shape. Flowers are yellow, sweet-scented, large, loose and as they age become brown-veinted. The fruits are long cylindrical, dark brown and hanging pod [11]. It is medicinal plant used traditionally in the treatment of various human ailments such as snakebites, bilharzias, skin diseases, cough, pneumonia, fever, gonorrhoea, abdominal pains, headaches and snakebites. In addition *C. abbreviata* extracts are used in treatment of water fever and heart diseases [12,13]. The medicinal efficacy of *C. abbreviata* is attributed to its phytochemical compounds most of which are phenolics, antioxidants and anthraquinones [14].

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C. abbreviata is used to treat skin rashes caused by human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) infections [15]. *C. abbreviata* also possess antidiabetic, antiviral, antihelminthic, antimalarial, antibacterial and antifungal and antioxidant activity [11].

Folklore information indicates that this plant is used in management of neoplasm. However, there is no documented scientific evidence for its antiproliferative activity. It is against this background that this study was designed to scientifically evaluate the unconfirmed antineoplastic activity of *C. abbreviata*.

Materials and Methods

Reagents

The reagents used in this study included; RPMI1640, Fetal Bovine serum, Phenol red, L-glutamine, HEPES, Trypsin, Sodium hydrogen carbonate, Hand gloves (sterile), Dimethyl sulfoxide (DMSO), 5 ml sterile graduated pipettes, MTT dye, Penicillin/Streptomycin, 96 well plate flat bottomed with cover, Culture flasks: T25, T75, Ethanol analar, Trypan Blue Sigma, 25 ml graduated sterile, pipettes, Eagles MEM medium, 24 well plate, Acetone and Pipette tips 20, 100, 200 µl. These reagents were procured from Sigma Aldrich, Germany. Tamoxifen, streptomycin and penicillin were procured locally from local pharmacies.

Equipment

The following equipment were used in this study; CO₂ Incubator (Sanyo, Japan), water bath (Sakura, Japan), Liophilizer (Edward, Britain), Electric Mill (Edward, Britain), Weighing Balance (Mettler AC 1000, Switzerland), Inverted Microscope (Nikon, Japan), Biosafety Cabinet Class II (Esco, Japan), Deep Freezer (Sanyo, Japan), ELISA plate reader (Labsystems, Japan) and Hemocytometer (Esco, Japan).

Cell lines

This study used HCC Hep G2, Macrophages Raw 264.7 and Vero E6 cell lines. All the cell lines were provided by Kenya medical research institute (KEMRI), centre for traditional medicine and drug research (CTMDR).

Collection and preparation of plant material

C. abbreviata root bark used in the study was collected from Bura, Taita Taveta with help of local herbalist. The root bark was cleaned and transported to KEMRI for further processing. The plant samples were then provided to an acknowledged taxonomist for botanical verification and a voucher specimen deposited at the KEMRI herbarium. The root bark was dried under shade at Kenya Medical Research Institute at room temperature. After drying it was ground to powder by an electric mill, weighed and packaged.

Aqueous extraction

Weight of 100 g of root bark powder of *C. abbreviata* was soaked in 1 liter of double distilled water and heated at 60°C in water bath for 6 hours for aqueous extraction. It was covered and left to cool under room temperature. The extract was decanted in a clean 1000 ml conical flask and filtered with Whatman no. 1 filter paper into a clean sterile 1000 ml conical flask. Decantation and filtration was repeated until the filtrate become clear. The filtrate was centrifuged at 3000 RPM for 5 minutes, freeze dried, weighed and stored in an air tight container under -20°C until future use [16,17].

Determination of antiproliferative activity

Cell culture: The cell lines used in the study were maintained as monolayer cultures in RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum (FBS), L-Glutamine, 100 µg/ml penicillin and 100 µg/ml streptomycin incubated at 37°C in a humidified incubator at 5% CO₂.

Cell treatment: The obtained cell monolayer was treated with trypsin 0.25%-EDTA 10 mM after they had reached 70-100% confluence. A volume of 5 ml of growth media was added to neutralize the trypsin enzyme. The cell suspension was centrifuged at 3000 rpm for 5 minutes. The supernatant was discarded and cells resuspended in 5 ml culture media and cell density was determined using trypan blue exclusion assay. The cells were seeded independently in 96 well plate at 2×10^4 cells per well at a volume of 100 µl and incubated at 5% CO₂ humidified incubator at 37°C for 24 hours.

The treatment was done by adding 50 µl of aqueous extract of *C. abbreviata* at 7 concentrations namely 1000 µg, 333.33 µg, 111.11 µg, 37.04 µg, 12.35 µg, 4.12 µg, 1.37 µg and the last row of the 96 well plates was left untreated. They were then incubated in 5% CO₂ humidified environment at 37°C for 48 h after which the MTT viability assay was carried out. The design is summarized in Table 1.

Viability MTT test

Principle: This is a colorimetric assay based on enzymatic activity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4,5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into formazan. Formazan is an insoluble yellow colored product that is measured spectrophotometrically [18,19]. The activity of the enzyme to produce formazan is directly proportional to the level of cell viability and inversely proportional to the level of cell inhibition [20,21].

MTT assay: The viability test was done by the tetrazolium 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide (MTT) dye reduction assay [18,22]. After 48 hours of incubation, the culture medium in plates was discarded and wells washed with phosphate buffered saline (PBS). A volume of 10 µl of PBS solution containing 10 µg/ml of MTT was pipetted into each well and the plates incubated for 3 hours. A volume of 10 µl of Pure DMSO was added and incubated for 30 minutes. The plates were mildly shaken at room temperature and the Optical density (OD) determined at 560 nm using a microplate reader spectrophotometer.

Determination of proliferation

The proliferation of the cells after treatment was calculated using a formula developed by Patel et al. [19] and Awasare et al. [23] as follows;

Groups	Treatment
	<i>Cassia abbreviata</i>
Negative control	Untreated cells
Experimental Groups	Concentration (µg/ml)
I	1.37
II	4.12
III	12.35
IV	37.04
V	111.11
VI	333.33
VII	1000

Table 1: Experimental design.

$$\text{Proliferation rate} = \frac{At - Ab}{Ac - Ab}$$

$$\text{Percentage viability} = \frac{At - Ab}{Ac - Ab} \times 100$$

$$\text{Percentage inhibition} = 100 - \frac{At - Ab}{Ac - Ab} \times 100$$

Where,

At=Absorbance value of test compound

Ab=Absorbance value of blank

Ac=Absorbance value of negative control (untreated cells)

Qualitative phytochemical screening

Qualitative phytochemical screening of aqueous root bark extract of *C. abbreviata* was performed as described by Algelaa and Osamudiamen [24]. Secondary metabolites tested for included; alkaloids, anthraquinones, flavonoids, phenols, saponins, and terpenoids.

Test for saponins: One gram of powdered roots of the plant was shaken vigorously with warm water in a test tube. After shaking the test tube was allowed to stand. A persistent froth was observed which indicated presence of saponins.

Test for tannins: Weight of 1 g of the plant extract was stirred in 2 ml of distilled water and the filtered. Ferric chloride was then added to the filtrate. A green precipitate was observed that indicated presence of tannins.

Test for alkaloids: Weight of 1 g of the plant extract was heated gently in 10 ml of 10% sulfuric acid for 5 minutes. Resulting solution was tested for presence of alkaloids by adding 2 drops of Meyer's reagent. There was no white precipitate formed which indicated absence of alkaloids

Test for phenolics: Weight of 1 g of the plant extract was dissolved in 2 ml of water and 2 drops of dilute ferric chloride solution was added. The formation of a red coloration evidenced presence of phenols.

Test for flavonoids (sodium hydroxide test): A weight of 1 g of the plant extract was dissolved in water, briefly warmed and filtered. Drop wise, 10% aqueous sodium hydroxide was added to 2 ml of the filtrate. This produced a yellow coloration which turned colorless upon drop wise addition of dilute hydrochloric acid which indicated presence of flavonoids.

Test for Anthraquinones: Weight of 2 g of the extract was added to 5 ml of benzene and filtered. Volume of 5 ml 10% ammonium hydroxide was added and shaken. Violet colour in the ammoniacal phase was evidence of anthraquinones presence.

Data management and statistical analysis

The data collected was both qualitative and quantitative. Data generated from *in vitro* antiproliferative potential of aqueous root bark extract of *C. abbreviata* experiments was quantitative data. It was stored in spreadsheets, subjected to descriptive statistics and values were expressed as Mean \pm SEM.

One-way ANOVA was used to compare means among treatment after which they were subjected to tukey's *post-hoc* test for pairwise separation and comparison of means. Values of $P \leq 0.05$ were considered

significantly different. Dose response curve was plotted and used to determined IC_{50} values by linear regression analysis. Unpaired t-test was used to compare between IC_{50} values of aqueous root bark extract of *C. abbreviata* and Tamoxifen, the reference drug. Analysis of the data was done using Minitab statistical software version 17.0. The data was presented in graphs and tables. Data generated from phytochemical screening of aqueous root bark extract of *C. abbreviata* was qualitative and was tabulated.

Results

Antiproliferative activity of aqueous root bark extract of *C. abbreviata* on HCC cell line

Generally, aqueous root bark extract of *C. abbreviata* inhibited proliferation rate of the HCC cell line. Table 2 shows the effects of aqueous root bark extract of *C. abbreviata* on proliferation rate of HCC cell line. There was a concentration dependent cell inhibition. As the concentration of aqueous root bark extract of *C. abbreviata* increased, the rate of cell proliferation decreased (Table 2 and Figure 1). Cell proliferation rate was lower at the extract concentration of 1000 $\mu\text{g/ml}$ compared to untreated cells. The highest cell proliferation rate was observed in untreated cells that was significantly different from all treatments of HCC cells with the aqueous root bark extract of *C. abbreviata* ($p < 0.05$; Table 2).

Similarly, as the concentration of aqueous root bark extract of *C. abbreviata* increased the percentage inhibition increased and percentage cell viability decreased (Figure 2).

Generally, it was observed that percentage cell inhibition was high at higher concentration and low at lower concentrations of the plant extract (Appendix I).

Antiproliferative activity of aqueous root bark extract of *C. abbreviata* on vero cell line

Treatment of vero cells with aqueous root bark extract of *C. abbreviata* generally reduced proliferation rate of the vero cells (Table 3 and Figure 3). The plant extract demonstrated a dose dependent inhibition of proliferation rate of vero cells. As the concentration of plant extract increased from 1.37 $\mu\text{g/ml}$ to 1000 $\mu\text{g/ml}$ the proliferation rate decreased. The proliferation rate was lowest at 1000 $\mu\text{g/ml}$ and the highest in untreated cells (Table 3 and Figure 3). There was a significant difference between proliferation rate at 1000 $\mu\text{g/ml}$ and untreated cells ($p < 0.05$; Table 3 and Figure 4) (Appendix II).

Percentage cell inhibition increased as the concentration of aqueous root bark extract of *C. abbreviata* increased while percentage cell viability increased as the concentration reduced. The highest percentage cell viability was observed in untreated cells and highest percentage cell inhibition was observed in 1000 $\mu\text{g/ml}$ (Appendix III) (Figure 4).

Antiproliferative activity of aqueous root bark extract of *C. abbreviata* on macrophage cell line

Administration of aqueous root bark extract of *C. abbreviata* to macrophage generally limited proliferation rate of the macrophage cells. The results of effect of aqueous root bark extract of *C. abbreviata* on macrophage cell line are shown in Table 4. The plant extract exhibited a dose dependent inhibition of proliferation rate of macrophage cell line. As the concentration of aqueous root bark extract of *C. abbreviata* enhanced, the proliferation rate of macrophage cells decreased (Table 5 and Figure 5). At 1000 $\mu\text{g/ml}$ the proliferation rate was lower compared to 1.37 $\mu\text{g/ml}$ of the plant extract. The proliferation rate was

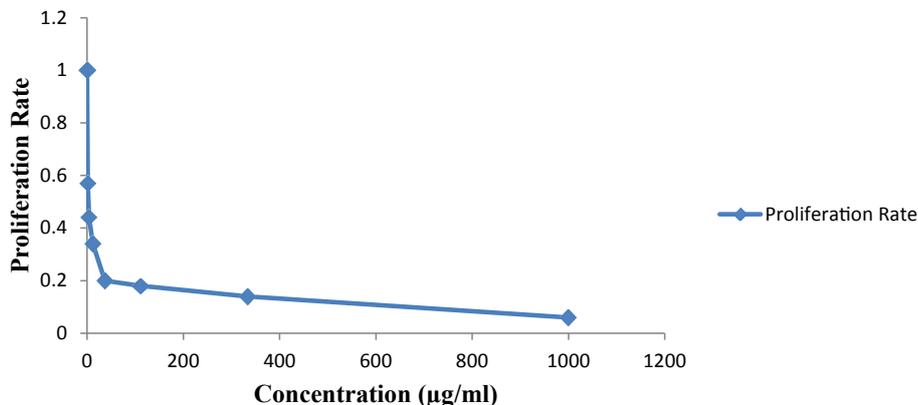


Figure 1: Dose response curve for effect of aqueous root bark extract of *Cassia abbreviata* on proliferation rate of HCC cell line.

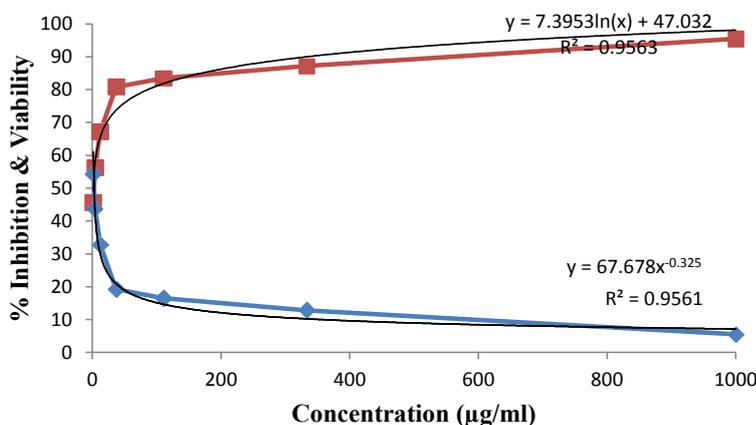


Figure 2: Dose response curve for percentage inhibition and percentage viability for effect of aqueous root bark extract of *Cassia abbreviata* on HCC cell line.

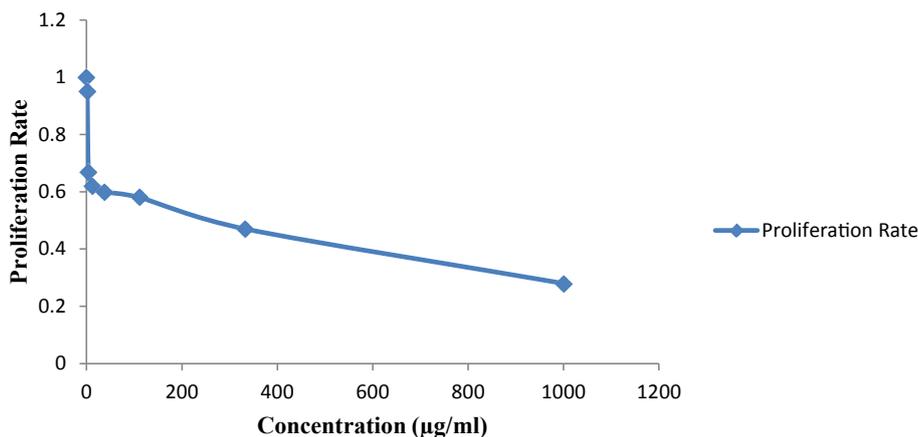


Figure 3: Dose response curve for effect of aqueous root bark extract of *Cassia abbreviata* on proliferation rate of vero cell line.

significantly different between treatments at 1000 µg/ml of plant extract and untreated cells ($p < 0.05$; Table 4).

High percentage cell viability was observed at lower concentrations of the plant extract (Appendix III) (Figure 6). As Figure 4.10 shows, there was an increase in percentage cell inhibition and decrease in percentage cell viability as the plant extract concentration increased.

Determination of IC_{50} of aqueous root bark extract of *C. abbreviata* against HCC, macrophage and vero cell lines

The IC_{50} values were determined from drug-response curve plotted on MS Excel using the equation of the trend lines attached to the curve. Aqueous root bark extract of *C. abbreviata* had IC_{50} of 1.49 µg/ml, 81.08 µg/ml, and 128.38 µg/ml on HCC, macrophage and vero cell lines

Concentration (µg/ml) <i>Cassia abbreviata</i>	Proliferation Rate
Untreated cells	1.00 ± 0.00 ^a
1.37	0.57 ± 0.01 ^b
4.12	0.44 ± 0.03 ^{bc}
12.35	0.34 ± 0.07 ^{bcd}
37.04	0.20 ± 0.04 ^{cde}
111.11	0.18 ± 0.04 ^{cde}
333.33	0.14 ± 0.06 ^{de}
1000	0.06 ± 0.04 ^e

All values are expressed as Mean ± SEM. Values followed by the same superscript are not significantly different (P>0.05).

Table 2: Effect of aqueous root bark extract of *Cassia abbreviata* on proliferation rate of HCC cell line.

Concentration (µg/ml) <i>Cassia abbreviata</i>	Proliferation Rate
Untreated Cells	1.00 ± 0.00 ^a
1.37	0.95 ± 0.00 ^a
4.12	0.67 ± 0.05 ^b
12.35	0.62 ± 0.02 ^{bc}
37.04	0.60 ± 0.02 ^{bc}
111.11	0.58 ± 0.02 ^{bc}
333.33	0.47 ± 0.01 ^c
1000	0.28 ± 0.08 ^d

All values are expressed as Mean ± SEM. Values followed by the same superscript are not significantly different (P>0.05)

Table 3: Effect of aqueous root bark extract of *Cassia abbreviata* on proliferation rate of vero cell line.

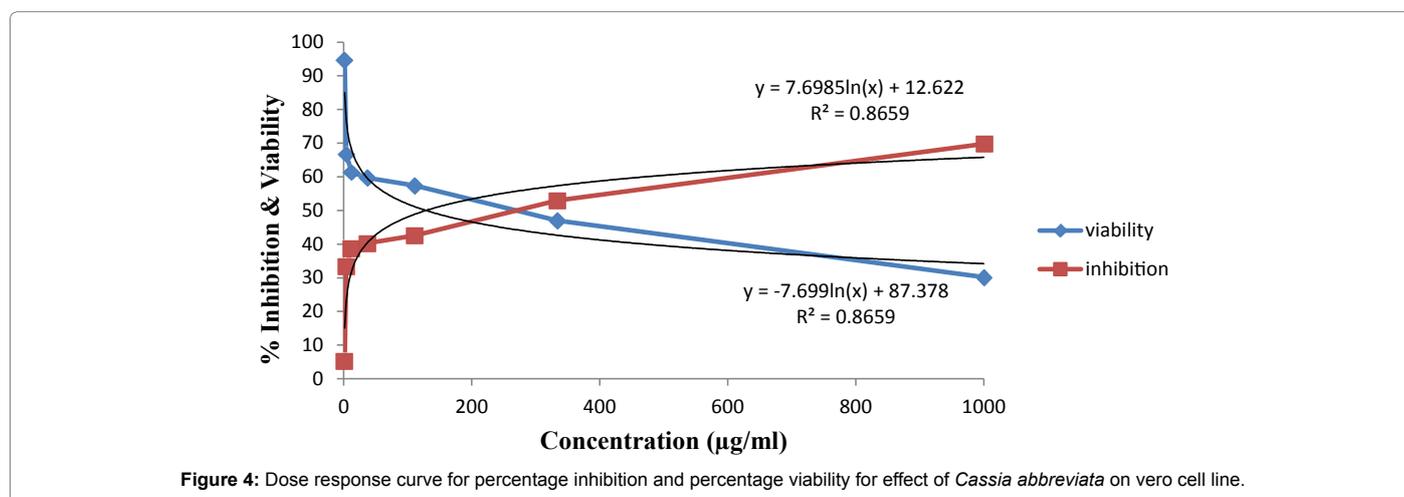


Figure 4: Dose response curve for percentage inhibition and percentage viability for effect of *Cassia abbreviata* on vero cell line.

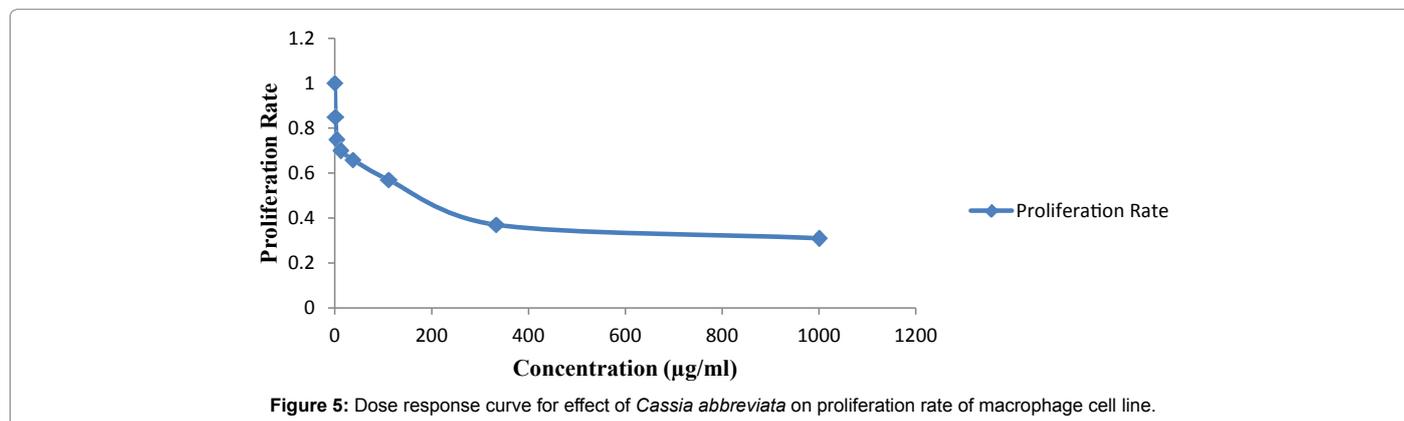


Figure 5: Dose response curve for effect of *Cassia abbreviata* on proliferation rate of macrophage cell line.

respectively. Aqueous root bark extract of *C. abbreviata* had the highest IC_{50} on HCC, followed by macrophage and vero cell lines.

Qualitative phytochemical screening

A number of classes of compounds were identified in aqueous root bark extract of *C. abbreviata*. As Table 6 shows the aqueous root bark extract of *C. abbreviata* contained flavonoids, saponins, anthraquinones, phenolics, tannins, saponins however alkaloids were absent.

Discussion

This research investigated the antiproliferative activity of aqueous root bark extract of *C. abbreviata* on three cell lines

namely; hepatocellular carcinoma (HCC), macrophage and vero cell lines. Generally, the aqueous root bark extract of *C. abbreviata* had antiproliferative activity against hepatocellular carcinoma, macrophages and vero cells. The plant extract demonstrated a concentration dependent antiproliferative activity on all the cell lines. A similar observation was made by Awasare et al. [23] who demonstrated a concentration dependent inhibition of five cell lines namely; human leukemia (HL-60) cell line, human colon cancer (HT-29) cell line, human breast cancer (MCF-7) cell line, human skin cancer (A 431) cell line and human lung cancer (A 549) cell lines by oleanane type of triterpenoid saponin from stem bark extract of *manilkara zapota* linn. Grbović et al. [24] observed a dose dependent inhibition of Colon

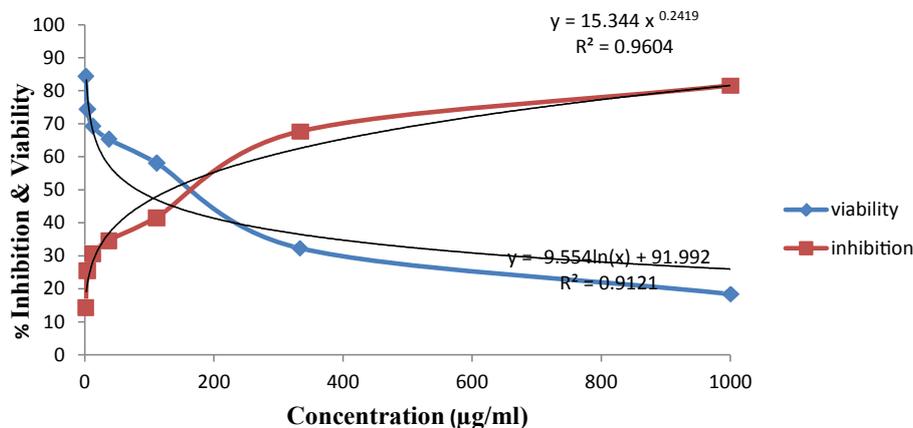


Figure 6: Dose response curve for percentage inhibition and percentage viability of effect of aqueous root bark extract of *Cassia abbreviata* on macrophage cell line.

Concentration (µg/ml) <i>Cassia abbreviata</i>	Proliferation Rate
Untreated Cells	1.00 ± 0.00 ^a
1.37	0.95 ± 0.00 ^a
4.16	0.67 ± 0.05 ^b
12.35	0.62 ± 0.02 ^{bc}
37.04	0.60 ± 0.02 ^{bc}
111.11	0.58 ± 0.02 ^{bc}
333.33	0.47 ± 0.01 ^c
1000	0.28 ± 0.08 ^d

All values are expressed as Mean ± SEM. Values followed by the same superscript are not significantly different (P>0.05)

Table 4: Effect of aqueous root bark extract of *Cassia abbreviata* on proliferation rate of macrophage cell line.

cancer (HCT-116) and breast cancer (MDA-MB-231) cell lines by methanolic extract of *Origanum vulgare* *in vitro*.

An extract is defined to be highly active if it has $IC_{50} < 10 \mu\text{g/ml}$, moderately active if the IC_{50} is between $10 \mu\text{g/ml}$ to $50 \mu\text{g/ml}$ and inactive if the $IC_{50} > 50 \mu\text{g/ml}$ [25]. In this study, the IC_{50} for *C. abbreviata* against HCC was $1.49 \mu\text{g/ml}$. Therefore, aqueous root bark extract of *C. abbreviata* is considered highly antiproliferative against the cancer cell line. On the other hand, the plant extract had an IC_{50} of $81.08 \mu\text{g/ml}$ and $128.38 \mu\text{g/ml}$ on macrophage and vero cell lines respectively. Its high IC_{50} values against normal vero and macrophage cell lines indicate that it is a safe anticancer medicinal plant and is selectively inhibitory on cancer cells. According to Zirich et al. [26] plants with $IC_{50} \geq 20 \mu\text{g/ml}$ are recognized as safe. Inhibitory selectivity of medicinal plants with antiproliferative activity has been also demonstrated in previous studies. Gul et al. [27] while determining antioxidant and antiproliferative activities of *Abrus precatorius* leaf extract, observed that the extract selectively inhibited proliferation of HCC cell lines among others while sparing macrophage cells. In a corresponding work, methanolic extracts of *Elaeis guineensis* have shown selective antiproliferative effects against MCF-7 and vero cell lines as demonstrated by Vijayarathna and Sasidharan [28].

The antiproliferative activity of aqueous root bark extract of *C. abbreviata* can be attributed to the phytochemicals present in the extract. Qualitative phytochemical screening showed that the aqueous root bark extract of *C. abbreviata* contains saponins, phenolics, anthraquinones, flavonoids and tannins. Tannins have been reported to possess

Cell Lines	IC_{50} <i>Cassia abbreviata</i>
HCC	1.49
Macrophages	81.08
Vero E6	128.38

Table 5: IC_{50} values of aqueous root bark extract of *Cassia abbreviata* and tamoxifen on HCC, macrophage and vero cell lines.

Phytochemicals	Results
Flavonoids	+
Phenolics	+
Tannins	+
Saponins	+
Alkaloids	-
Anthraquinones	+

Present phytochemicals denoted by (+) sign; absent phytochemicals denoted by (-) sign.

Table 6: Qualitative phytochemistry of aqueous root bark extract *Cassia abbreviata*.

anticancer and antioxidant activities [29,30]. A tannin compound, Cuphiin D1 (CD1), isolated from *Cuphea hyssopifolia* has been proven to exert antiproliferative effects against human promyelocytic leukemia HL-60 cell line [31]. Corilagin, a tannin compound, isolated from a number of medicinal plants including *Phyllanthus niruri* has been shown to have antiproliferative effects against ovarian cancer cell lines [32]. Pancreatic cancer cells and breast cancer cells proliferation has been inhibited by Ellitannin compound isolated from the *Cistus ladanifer* [33].

Saponins have also been reported to show antioxidant and anticancer effects [34]. It has been observed that triterpene saponins (saxifragifolin B and saxifragifolin D) extracted from *Androsace umbellata* extract inhibited cancer cell growth and induced apoptosis in multi-drug resistance and non-multidrug resistance tumor cell lines [35]. Hu et al. [36] found that triterpene saponins extracted from *Nepenthes glandulifera* exhibited antiproliferative activity against human lung carcinoma A-549 cell line.

Flavonoids have been shown to possess antioxidant and anticancer properties [37,38]. Flavopiridol is a flavonoid that has been shown to inhibit several cyclin dependent kinases blocking the cell cycle block.

It has demonstrated anticancer activity in renal, colon, non-Hodgkin's lymphoma, prostate, colon and gastric carcinomas [39]. Quercetin, a flavonoid compound has been reported to have antiproliferative activity against many cancers namely hepatocellular carcinoma and neuroblastoma [40,41]. Therefore, it is likely that the phytochemicals identified as occurring in the aqueous root bark extract of *C. abbreviata* are responsible for its antiproliferative activity. There are number of possible mechanisms of activity that antiproliferative effects of the plant extract could be postulated. Such mechanisms include cell cycle arrest, apoptosis and inhibition of transcriptional proteins. The plant antiproliferative mechanism of activity should be empirically determined since this study did not.

Therefore, *C. abbreviata* serves as a good bio-resource for generating a readily available herbal anticancer formulation. This study, therefore, scientifically confirms and supports the traditional use of *C. abbreviata* for management of neoplasm. However, the antiproliferative mechanism of action of the extract is still obscure. There a need to confirm the actual mechanism of action of the extract. The plant extract should be screened for antiproliferative activity and safety *in vivo*. Moreso, phytochemicals quantification should be done. Consequently isolation and characterization of pure compound with anticancer activity from the extract should be done.

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