

FULL ARTICLE

Hydro-ethanol seed extract of *Theobroma cacao* exhibits antioxidant activities and potential anticancer property

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Abstract

Despite the nutritional value of *Theobroma cacao* seed (cocoa), the antiproliferative activity is yet to be fully elucidated. Therefore, the current study investigated the antioxidant and potential anticancer activities of the seed extract. The *in-vitro* free radical scavenging activity was evaluated via DPPH, nitric oxide, lipid peroxidation, and reducing power activity. The effect of the cocoa extract on mitotic cell division was determined using *Allium cepa* assay. The phytochemical screening of the seed extract revealed the presence of cardiac glycoside, phenol, tannin, steroid, terpenoid, alkaloid, saponin, and flavonoid at the following concentrations; 31.21 ± 0.57 , 29.16 ± 0.43 , 18.51 ± 0.06 , 9.57 ± 0.08 , 23.77 ± 0.08 , 6.58 ± 0.04 , 28.65 ± 0.13 , and 22.16 ± 0.18 mg/100 g, respectively. The *A. cepa* assay revealed a significant influence of the extract on mitotic cell division in a concentration dependent manner, the antiproliferative effect suggests a potential anticancer property.

Practical applications

The Cocoa plant has been of great nutritional benefit over the years. The seeds (*Theobroma cacao*) is used for making varieties of chocolate food beverages, chocolate candies, confectioneries, and food thickening, however, the anticancer activity is still an area of deliberation. *Theobroma cacao seed extract* scavenged diphenyl-2-picrylhydrazyl radical, nitric oxide, lipid peroxidation at $50.03 \pm 1.48\%$ at a concentration of $60 \mu\text{g/ml}$, $50.84 \pm 0.88\%$ at $40 \mu\text{g/ml}$, and $50.79 \pm 0.37\%$ at $40 \mu\text{g/ml}$, respectively. The reducing power activity was 0.329 ± 0.001 per $100 \mu\text{g/ml}$. The Gas Chromatography Mass Spectroscopy analysis showed the most abundant compound to be caffeine (98.09%). Mild quantities of hexa-decanoic acid, methyl extract were also detected. The hydro-alcohol extract of *Theobroma cacao seeds* exhibited high antioxidant and antimitotic activities, suggesting a possible cytotoxic potential against abnormal cell growth, such as cancer. This study substantiates its ethno-medicinal use in the prevention and control of cancer.

KEYWORDS

Allium cepa assay, anticancer, antimitotic, antioxidant, GCMS, *Theobroma cacao* seed

1 | INTRODUCTION

One of the most devastating health threatening disease of this era is cancer, whose clinical research attempts have experienced failures over time (Begley & Ellis, 2012). It is a disease of abnormal cell growth in which normal cells are converted to malignant masses which divide in an uncontrollable manner. Its occurrence is multifaceted in nature. Cancer could occur due to the effect of harmful chemicals, viruses, free radical producing agents, some environmental toxins and inevitable routine life factors. Traditional treatment of cancer, such as chemotherapy, radiotherapy, and surgery provide only incomplete and momentary relief. New pharmacological approaches for cancer drug discovery that focuses on whole-animal testing, chemistry and genetics are also being developed (Dar, Das, Shokat, & Cagan, 2012). However, treatments and synthetic anticancer drugs are costly and beyond the reach of the general public, coupled with the several failures of treatments and clinical trials (Begley & Ellis, 2012). Hence, alternative herbal remedies that are commonly available and comparatively economical, with no side effects are currently being explored. There are many factors which affect the causes and aggravation of this disease, this include factors that induces oxidative stress within the cells.

Nutrition influences cancer causation, prevention, and control to a large extent. Phytoconstituents which enhances prevention and control of cancer have been reported, most especially phyto-polyphenols, such as resveratrol (Chen, Ganapathy, Singh, Shankar, & Srivastava, 2010). Recently, reported studies revealed the potential protective effect of moderate consumption of chocolate on cardiovascular risk (Gianfredi, Salvatori, Villarini, & Moretti, 2018).

Theobroma cacao seeds (TC), commonly consumed in cocoa beverages and chocolate, have been considered as a polyphenol rich food, such as flavonoids and phenolic acids, which are the main forms of polyphenols. Cocoa is a potential source of more phenolic phytochemicals per serving than tea and red wine. This enhances its antioxidant and anti-inflammatory activities (Kim, Lee, & Hyong, 2011). The potential health implications of biologically active substances present in its components are well documented (Liebert et al., 2000). Many epidemiological studies associate cocoa and chocolate consumption to a reduced risk of chronic diseases, and various health benefits of the cocoa compounds, have been attributed to its antioxidant and anti-inflammatory potency (Ellam & Williamson, 2013). In particular, the bioactive constituents of cocoa components exhibit pharmacologic effects in reducing inflammatory processes (Solà et al., 2012). This is as a result of their ability to down regulate pro-inflammatory cytokines and their downstream biochemical pathways, cocoa equally possesses cardiovascular health benefits (Corti, Flammer, Hollenberg, & Lüscher, 2009).

Recent studies investigating the chemotherapeutic potential of medicinal plants have been carried out to discover new therapeutic agents or complementary alternative medicines, that lack the toxic side effects of chemotherapeutic drugs, with the same or better curative effects associated with the current synthetic therapeutic agents (Mahavorasirikul, Viyanant, Chaijaroenkul, Itharat,

& Na-bangchang, 2010; Venkateshwar, Rao, Sateesh, Mujahidul, & Mansour, 2008). During the early discovery stages, plant-based anticancer compounds were evaluated by bioassay screening methods, such potential anticancer compounds are investigated to clarify their mechanism of action against various cancer cells (Chen et al., 2010; Liu, 2004; Lu, 2003).

The cocoa bean contains numerous phytochemicals. The consumption of cocoa, a polyphenol-rich food chocolate and its by-product, which have high antioxidant activity and anti-inflammatory effect, could be beneficial in decreasing the damage caused by genotoxic and epigenetic carcinogens. They equally inhibit the complex processes leading to cancer (Ellam & Williamson, 2013; Kim et al., 2011; Liebert et al., 2000). Based on the known facts, the present study made some further investigation into the potential anticancer property of the *Theobroma cacao* seed extract.

2 | MATERIALS AND METHODS

2.1 | Chemicals and Reagents

All the chemicals and reagents used in the present study are of analytical grade. Equipment used were calibrated and in a good working condition. Some of the reagents and equipment used are eighty percent (80%) Ethanol solution, Petroleum ether, 1.25% Na₂SO₄, 1.25% H₂SO₄, Ethanol, 0.4 M Phosphate buffer and Biuret solution, 1% aqueous hydrochloric acid, Olive oil, diluted ammonia solution, H₂SO₄, Acetic anhydride, Chloroform, Glacial acetic acid, 0.1% Ferric chloride solution, Sulfuric acid, Acidified ethanol, Mayer's reagent, Drangendroff's reagent, FeCl₃, HCl, Pottasium ferrocyanide, Folin-Ciocalteau reagent, 75% sodium carbonate solution, Methanol, 10% Aluminum Chloride, 1 M Potassium acetate, Quercetin solution, 0.6 M Sulfuric acid, 28 mM Sodium Phosphate, 4 mM ammonium molybdate, 95% ethanol, DPPH solution, Liver homogenate, Tris-HCl buffer, FeCl₂, Ascorbic acid, HCl, Malondialdehyde (MDA), Trichloroaceticacid (TCA), Thiobarbituric acid (TBA), Sodium Nitroprusside, Griess reagent, Ethanol, Sodium suldate, Silica gel, Cotton wool, Gas Chromatography Mass Spectroscopy (GCMS) analyzer, Acetic acid, ethanol, HCl, Microscope, and 2% Orcein Solution.

2.2 | Sample collection and preparation

Crude cocoa extract was prepared according previously established protocol by Ruzaidi, Abbe Maleyki, Amin, Nawalyah, and Muhajir (2005). Cocoa seeds were purchased from Ondo State, western part of Nigeria. The cocoa seeds were air dried under a shade and grounded into fine powder.

2.3 | Preparation of hydro-alcoholic extract of *Theobroma cacao*

The crude extract was prepared by mixing dried powdered cocoa seeds (40 g) with 80% (v/v) ethanol for 2 hr in the ratio of 1:10. The ethanol was removed completely from the extract using a rotatory

evaporator (Buchi Rotavor R-200) for 40 min at 55°C. The resulting aqueous cocoa extract was dried using an evaporator dish. The dried cocoa seed extract was stored at 4°C for further analysis.

2.4 | Proximate analysis

Proximate analysis of the *Theobroma cacao* sample was carried out to determine the amount of moisture content, total protein, fat content (ether extract), ash content, total fiber, and nitrogen-free extract of the *Theobroma cacao* sample.

2.5 | Phytochemical Screening

Qualitative phytochemical screening was carried out on the aqueous extract using standard procedures to identify the bio-active constituents present. The extract was tested for the presence of tannins, phlobatannins, saponin, flavonoids, steroids, terpenoids (Salkowski test), cardiac glycosides (Keller–Killani test), alkaloid (Mayer's reagent and Dragendroff's reagent).

2.6 | Estimation of Phytochemical Constituent

2.6.1 | Estimation of tannins

Tannin was estimated by dissolving 500 mg sample of the concentrate in 50 ml of distilled water, mixture was shaken for 1 hr. A 5 ml aliquot of the filtrate was mixed with 2 ml of 0.1 M FeCl₃ in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 720 nm within 10 min.

2.6.2 | Estimation of total phenolic compound

The hydro-ethanol seed extract, 0.5 grams was dissolved in 50 ml of distilled water. Exactly 0.5 ml of the solution was added to 0.1 ml of Folin–Ciocalteu reagent (0.5 M) mix and incubated at room temperature for 15 min, 2.5 ml sodium carbonate solution (7.5% w/v) was added afterward and the solution was further incubated for 30 min at room temperature. The absorbance of the solution was measured at 760 nm. The concentration of total phenol was expressed as gallic acid equivalent (mg/g of dry mass).

2.6.3 | Total flavonoid content estimation

One ml of sample solution (100 µg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% Aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. The resulting mixture was incubated at room temperature for 30 min and the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol.

2.7 | Antioxidant assay

Total antioxidant capacity, DPPH radical scavenging activity, nitric oxide scavenging activity, and *in vitro* lipid peroxidation assay were determined according to standard procedures.

2.7.1 | Total antioxidant capacity determination

One milliliter of the extract solution was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium-molybdate) in some test tubes. The tubes were capped and incubated in a boiling water bath at 100°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm. The total antioxidant capacity was expressed as equivalent of ascorbic acid.

2.7.2 | DPPH radical scavenging activity assay

An aliquot of 0.5 ml of extract in ethanol (95%) at different concentrations (25, 50, 75, and 100 µg/ml) were mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample, while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 min, the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm with the aid of a spectrophotometer. The scavenging effect was calculated using the expression:

$$\% \text{ inhibition} = \left[\frac{A_0 - A_1}{A_0} \right] \times 100$$

where A₀ is the absorption of the blank sample and A₁ is the absorption of the extract.

2.7.3 | *In vitro* lipid peroxidation assay

The reaction mixture containing liver homogenate (0.2 ml), Tris–HCl buffer (20 mM, pH 7.0, 0.1 ml), FeCl₂ (2 mM, 0.1 ml), ascorbic acid (10 mM, 0.1 ml), and 0.5 ml plant extract (25–100 µg/ml) in a final volume of 1 ml. The reaction mixture was incubated at 37°C for 1 h. Lipid peroxidation was measured as MDA using TCA, TBA, and HCl (TBA–TCA reagent: 0.375% w/v TBA; 15% w/v TCA and 0.25 M HCl). The incubated reaction mixture was mixed with 2 ml of TBA–TCA reagent and heated in a boiling water bath for 15 min. After cooling, the flocculent precipitate was removed by centrifugation at 10,000 g for 5 min. Finally, MDA concentration in the supernatant fraction was determined spectrophotometrically at 535 nm. Ascorbic acid was used as standard.

2.7.4 | Nitric oxide scavenging activity assay

Four (4) ml sample of plant extract, in standard solution at different concentrations (25, 50, 75, and 100 µg/ml) were taken in different test tubes and 1 ml of Sodium nitroprusside (5 mM in phosphate buffered saline) solution was added into each test tube. They were incubated for 2 hr at 30°C to complete the reaction.

Two (2) milliliters of sample were withdrawn from the mixture with 1.2 Griess reagent (1% Sulfanilamide, 0.1% of naphthyl-ethylene-di-amine-di-hydrochloride in 2% H₃PO₄).

TABLE 1 Proximate composition of *Theobroma cacao* extract. Values represent mean \pm SD. Dunnett's multiple comparisons test ($n = 5$, $p < 0.05$) when compared to the moisture content

Constituent	Composition (%)
Moisture	3.73 \pm 0.19
Ash	0.20 \pm 0.08***
Crude fat	34.74 \pm 1.96****
Crude fiber	4.01 \pm 0.46
Crude protein	10.05 \pm 0.92****
Carbohydrate	47.72 \pm 2.18****

*** and **** shows the level of statistical significance with respect to the control, $p < 0.001$ and 0.0001 , respectively.

The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and its subsequent coupling with naphthylethylenediamine were measured at 550 nm. Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation: $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of extract or standard.

2.8 | Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry was performed with the aid of a GC-MS machine: Agilent Technologies 7890A GC coupled to an Agilent Technologies 5575C. The analysis was carried out using the mobile phase, the carrier gas (helium) and the stationary phase, Chirasil-DEX capillary columns (30 m length, 0.32 mm internal diameter and 0.25 μ m film thickness-Chrompack).

Two grams (2 g) of the crude sample was weighed in a conical flask, 10 ml of ethanol was added to it and the mixture was placed on a shaker for 2 hr. The mixture was then placed in an ultrasonic bath and extraction was done for 3 hr. The mixture was allowed to cool at room temperature and was filtered through a column containing anhydrous sodium sulfate, silica gel, and cotton wool was used as a filter medium. The extract was allowed to concentrate in an open air fume cupboard to 2 ml. The GC-MS was performed on the extract. GC conditions were as follows: Oven temperature increased from 60 to 1808°C, at 108°C/min after an initial hold at 608°C for 3 min; Injector and detector temperature: 2,308°C. MS conditions were as follows: Ion source temperature: 2,408°C; the electron impact was: 70 eV; acquisition mode: scan (m/z 40–400).

2.9 | In vitro Assessment of Antimitotic Activity (Allium Assay)

The antimitotic activity of the extract was evaluated using *Allium cepa* root meristematic cells. *A. cepa* was sprouted in tap water for 72 hr at room temperature. The bulbs that developed with uniform roots were used for the experiment (2–3 cm length). These roots were treated with the sample extracts in three concentrations of

100%, 75%, and 50%. Distilled water was used as medium for dilution. A blank with distilled water was used as control (0%). After 24 hr of treatment, the root tips were fixed with fixing solution of acetic acid and alcohol (1:3) and hydrolyzed by boiling with hot HCl for 10 min at 60°C. Squash preparations were made by staining the treated roots with 2% Orcein solution. The mitotic index was calculated as.

$$\text{Mitotic Index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

2.9.1 | Statistical analysis

Analysis of the results were presented as mean \pm SD, there were three replicates and the data were analyzed by one or two-way analysis of variance (ANOVA) using Graphpad prism statistical software version 6. Significant differences between means were determined and analysis were considered significant if $p < 0.05$.

3 | RESULTS

3.1 | Proximate Composition

Theobroma cacao seed extract composed of carbohydrate, crude fat, crude protein, crude fiber, ash and moisture at 47.72 \pm 2.18, 34.74 \pm 1.96, 10.05 \pm 0.92, 4.01 \pm 0.46, 0.20 \pm 0.08, and 3.73 \pm 0.19%, respectively, as shown in Table 1. The nutritional composition of the extract revealed an appreciable amount of dietary nutrients, therefore, this study validates the use of *Theobroma cacao* seeds in beverages and food drinks for nourishing purposes.

3.2 | Phytochemical Screening

Theobroma cacao seed extracts revealed the presence of flavonoids, alkaloids, tannins, steroid, cardiac glycoside, terpenoids, anthraquinone, saponin, and phenol as presented in Table 2.

3.3 | Estimation of Phytochemical constituent

The various concentrations of the phytochemical constituent were determined (Table 2). *Theobroma cacao* extract was high in cardiac glycoside with a concentration of 31.21 \pm 0.57 mg/100 g and phenol with a concentration of 29.16 \pm 0.43 mg/100 g. Other constituents include tannin, steroid, terpenoids, alkaloid, saponin, and flavonoid with concentrations of 18.51 \pm 0.09, 9.57 \pm 0.08, 23.77 \pm 0.08, 6.58 \pm 0.04, 28.65 \pm 0.13, and 22.16 \pm 0.18 mg/100 g, respectively.

3.4 | Antioxidant Assay

In order to ascertain the free radical scavenging power of *Theobroma cacao* seed extract, the antioxidant activity was evaluated using DPPH free radical scavenging, nitric oxide scavenging, lipid peroxidation and reducing power activities. Each sample was prepared in a concentration of 20, 40, 60, 80, and 100 μ g/ml. The

TABLE 2 Quantitative phytochemical composition of *Theobroma cacao* extract. Values represent mean \pm SD. Tukey's multiple comparisons test ($n = 5$, $p < 0.0001$)

Phytochemicals	Concentration (mg/100 g)
Tannin	18.51 \pm 0.06
Steroid	9.57 \pm 0.08
Cardiac glycoside	31.21 \pm 0.57
Terpenoids	23.77 \pm 0.08
Alkaloid	6.58 \pm 0.04
Saponin	28.65 \pm 0.13
Flavonoid	22.16 \pm 0.18
Phenol	29.16 \pm 0.43

result revealed the potent antioxidant activities of the seed extract, as evident in the dose dependent scavenging effect (Figure 1a), the nitric oxide scavenging activity also revealed a dose-dependent effect (Figure 1b). The *in-vitro* lipid peroxidation assay revealed an increase in lipid peroxidation in the liver homogenate samples (Figure 1c). The *Theobroma cacao* seed extract equally exhibited a potent reducing power activity in a dose dependent manner (Figure 1d). These observations may be as a result of the potent antioxidant phytoconstituents present in the seed extract.

3.5 | Gas Chromatography-Mass Spectrometry

Gas Chromatography-Mass Spectrometry revealed the chemical constituent of *Theobroma cacao* seed extract (Figure 2). Caffeine was seen to be the most abundant with a total of 99.08% obtained

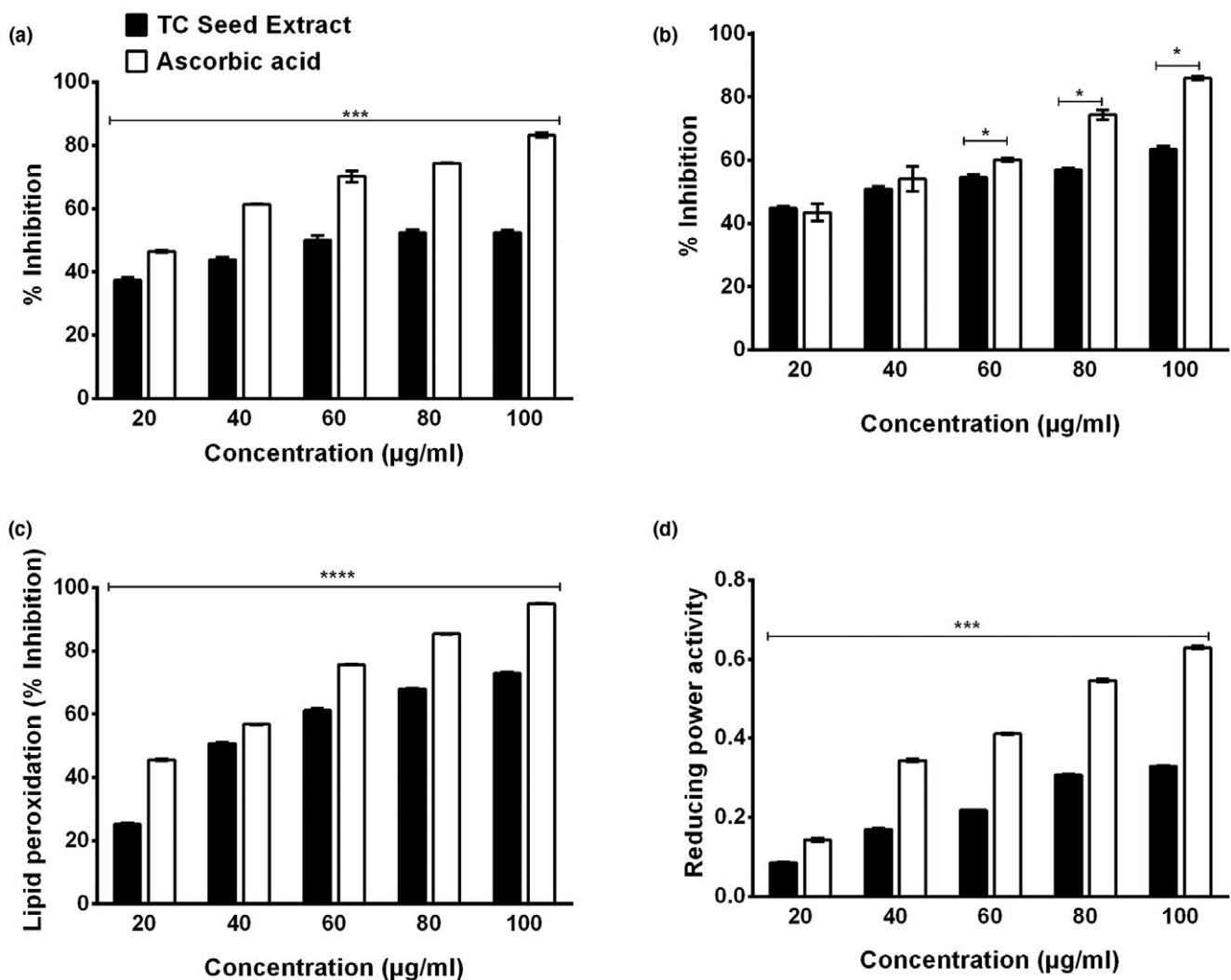


FIGURE 1 Antioxidant and free radical scavenging activity of *Theobroma cacao* seed extract in a dose-dependent manner. (a) DPPH free radical scavenging activity. Percentage inhibition of 2,2,-diphenyl-1-picrylhydrazyl (DPPH free radical) by hydro-ethanol seed extract of *Theobroma cacao* and standard ascorbic acid, (b) nitric oxide scavenging activity. Inhibition of nitric oxide radical by hydro-ethanol seed extract of *Theobroma cacao* and standard ascorbic acid, (c) lipid peroxidation scavenging activity. Percentage inhibition of lipid peroxidation by hydro-ethanol seed extract of *Theobroma cacao* and standard ascorbic acid, (d) reducing power activity. The reducing power activity of hydro-ethanol seed extract of *Theobroma cacao* and standard ascorbic acid. Analysis was done in triplicate, mean \pm SD. Two-way ANOVA ($n = 3$, $p < 0.0001$)

Abundance

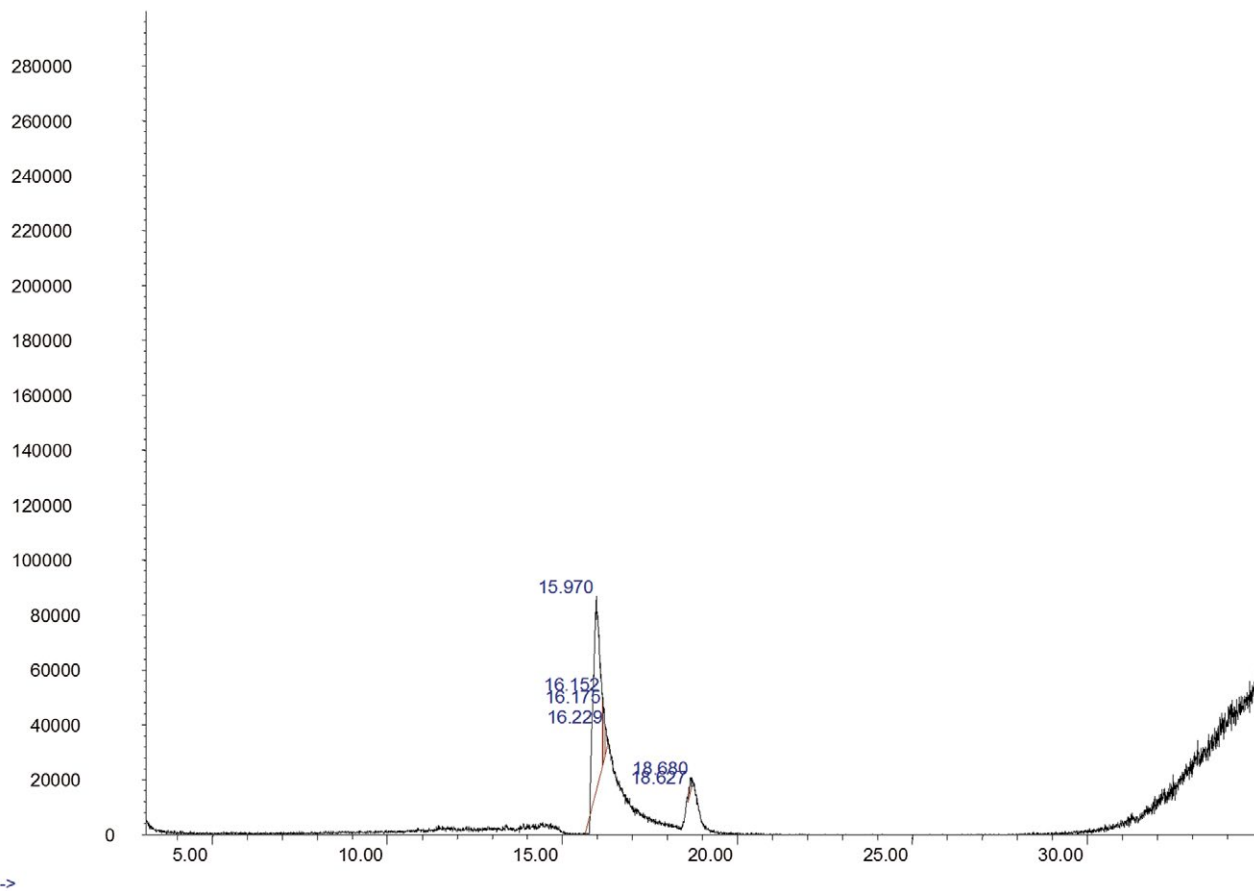


FIGURE 2 GCMS resolutions of *Theobroma cacao* extract constituents

at 15.970, 16.152, 16.175, and 16.229 min. The fatty acids, hexadecanoic acid and methyl ester were also detected at an abundance of 1.91% at 18.627 and 18.680 min. Other compounds detected are 4-dimethyl-4, 5, 7, 8-tetrahydroimidazo-[4, 5-E]-1, 4-diazepin-5, 8(6H)-dione and pentalene, octahydro at 15.970 and 16.152 min, respectively, (Table 3). The GCMS spectrum of hexadecanoic acid and caffeine are shown in Figures 3 and 4, respectively.

TABLE 3 GCMS dominant compounds at different retention time

Retention time (min)	Dominant compound	Dominant compound (%)
15.970	Caffeine	98.09%
16.152		
16.175		
16.229		
18.627	Hexa-decanoic acid, methyl ester	1.91%
18.680		

3.6 | Mitotic Cell Division in *A. cepa* Assay

The effect of test extract of *Theobroma cacao* seed was studied on root growth and mitotic index. The result indicated cocoa seed extract has significant influence on mitotic cell division of *A. cepa* root (Figure 5). *Theobroma cacao* seed extract at the concentration of 50%, 75%, and 100% exhibited significant reduction ($p < 0.001$) in mitotic index in a concentration dependent manner compared to control group (Table 4). This observation revealed the antiproliferative property of the extract, which could be an anticancer property.

4 | DISCUSSION

Prevention of cancer is the best form of control, since there is presently very few effective chemotherapeutic measures. The ethnomedicinal importance of *Theobroma cacao* seeds in general health is an established fact, however, the role in the prevention of cancer is still an area of deliberation. There is an increase in awareness, especially the role of antioxidants in the mediation of oxidative stress, which is the main underlying cause of various diseases of mankind

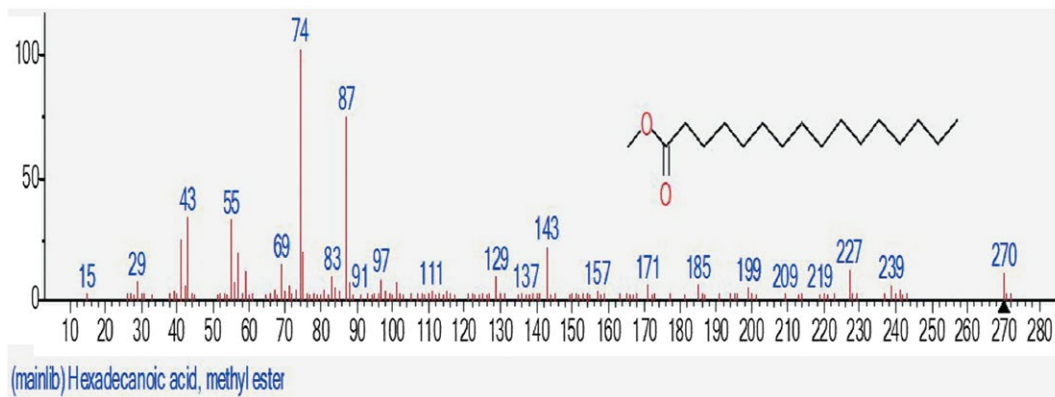


FIGURE 3 GCMS spectrum of hexa-decanoic acid, methyl ester with x-axis m/z ratio and y-axis as intensity

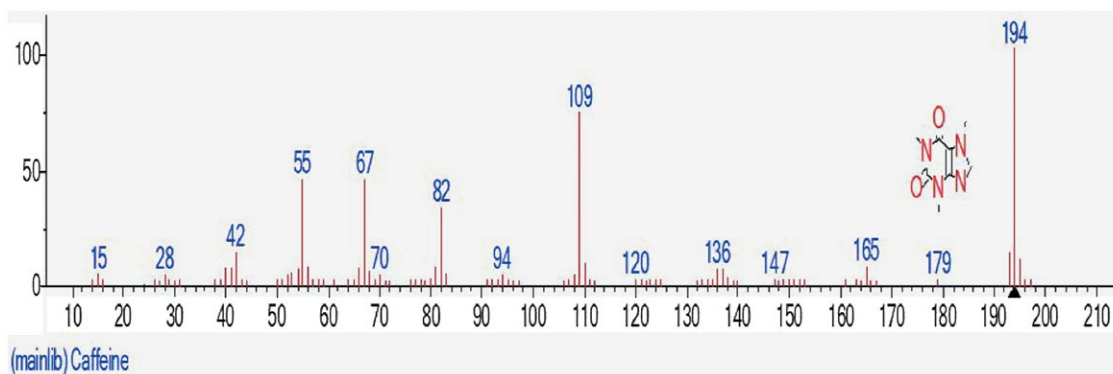


FIGURE 4 GCMS spectrum of Caffeine with x-axis m/z ratio and y-axis as intensity

including cancer (Bjørklund & Chirumbolo, 2017). The health benefits of phytoconstituents, such as bioflavonoids which is a potent antioxidant, has been shown in the prevention and control of oxidative stress induced by environmental pollutants and contaminants (Abolaji, Babalola, Adegoke, & Farombi, 2017). *Theobroma cacao* (Cocoa) is a plant with a wide range of broad spectrum of nutritional and medicinal activities, the plant possesses a wide variety of nutritional and therapeutic values. The major phyto-constituents of the plant are flavonoids, alkaloids, saponins, sterols, tannins, quinines, phenolic compounds like baicalein, oroxylym A and their glycosides (Kim, Lee, & Hyong, 2011, Liebert et al., 2000). Many of these compounds have shown potency in arresting the cell growth and

proliferation, and thus they provide new challenges in discovering the anticancer drugs from plant origin (Shachi, 2012).

The success rate of current chemotherapies are very slim, hence, the need to identify new compounds that will be efficient in cancer therapies, with little or no side effects from phytoconstituents of natural herbs (Kurangi & Jalalpure, 2018). In order to evaluate the anticancer potential of the seed extract of cocoa, the present study examined the antioxidant and antimetabolic activities of the hydro-ethanol seed extract of *Theobroma cacao*, in view of its possible medicinal use in the treatment and prevention of cancer conditions, while taking into consideration the general fact that the plant exert anticancer action through inhibition of cell division. The observed

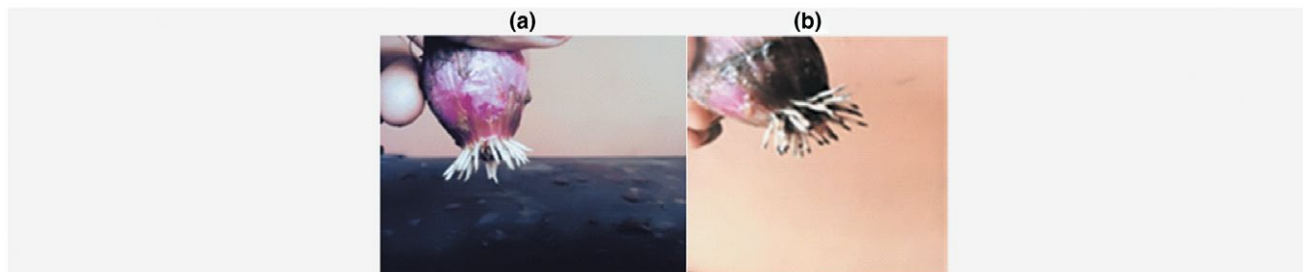


FIGURE 5 Effect of *Theobroma cacao* extract on *Allium cepa* bulb roots. (a) Non-treated onion roots, (b) onion roots after treatment with 100% *Theobroma cacao* extract

TABLE 4 *Theobroma cacao* extract mitotic index. There was a significant decrease in mitotic index with increase in extract concentration ($p < 0.05$)

Extract	0% (Water)	50%	75%	100%
Total no. of cells	524	498	508	485
Prophase	202	104	82	66
Metaphase	130	93	33	13
Anaphase	98	70	24	8
Telophase	34	22	12	3
Total no. of dividing cells	465	291	151	91
Mitotic index (%)	88.74	58.43	29.72	18.76

antimitotic property of the extract may not necessarily substantiate the anticancer activity of the plant, however, we have shown the potential of the plant to exhibit antiproliferative property subject to further research.

Phytochemical screening of the cacao seed extract revealed the presence of flavonoids, alkaloids and phenols that are known to have anticancer properties, this is in line with previous results of Ellam and Williamson (2013). The cancer preventive effect of several fruits and vegetables have been reported to be due to the presence of high content of antioxidants, such as flavonoid and phenolic compounds present in them. Most citrus fruits are rich source of citrus bioflavonoid which have been reported to possess anticancer effects (Block, Patterson, & Subar, 1992). The phytochemical quantitative estimation revealed an appreciable amount of flavonoids and phenolic phytoconstituents in *Theobroma cacao* seed extract, this is an indication of the high antioxidant content of the extract.

In order to ascertain the free radical scavenging power or antioxidant capacity and the reducing power activity of the seed extract, the antioxidant activity was done using DPPH, Nitric oxide and lipid peroxidation measures. The antioxidant activity correlated with the content of extract, which correlated with the content of phenolic compounds in investigated product (Figure 1). The present result is in line with previous studies which stated that certain phenolic containing plants provide oxidative stress resistant effects, thereby preventing oxidative damage, most especially epicatechins (Bernatova, 2018). Other studies equally revealed the free radical power of the glutelin fraction of the cocoa seed extract (Tovar-pérez, Guerrero-becerra, & Lugo-cervantes, 2017). The biochemical pathway of oxidative stress induced by free radicals, is highly implicated in the origin of degenerative diseases and some cancers, these free radicals are equally by-products of normal cellular metabolism, continuously inevitably produced in humans during aerobic respiration. However, when the antioxidant defense system cannot counteract the effects of these free radicals, the body tend to be in a biochemical state of oxidative stress (Guerra-Araiza et al., 2013; Kregel & Zhang, 2007;

Sohal & Orr, 2012). Frequent consumption of cocoa based food has been reported to offer antioxidant and anti-inflammatory properties, thereby offering protective effects against oxidative stress. Epicatechin-containing food, such as cocoa, has been reported to have blood pressure lowering effect, and their mechanism of action is in their ability to prevent oxidative damage and endothelial dysfunction (Bernatova, 2018).

In order to evaluate the antimitotic activity of the cocoa seed extract, this was carried out using *A. cepa* root meristem model, commonly known as *Allium* assay. Root meristematic cells were used for screening of drugs with antimitotic activity (Fiskesjo, 1985). In meristematic region, the cell division is similar to cancer cell division in humans. Therefore, these meristematic cells can be evaluated for screening of drugs with potential antimitotic activity. *Allium* assay is considered a rapid, highly sensitive, and reproducible bioassay for detecting cytotoxicity and genotoxicity. The root growth inhibition and antimitotic effects provide the indication of genotoxicity. The good genotoxic assay performance of *A. cepa* as a plant system has been attributed to the easily studied karyotype of plant [$2n = 16$] and the ability to correlate outcomes of assays with those of mammalian cells in the course toxic evaluations. The *A. cepa* species commonly known as onion, being characterized by homogenous meristematic cells and very large sixteen number chromosomes are ideal for use in bioassays (Shachi, 2012).

Antimitotic drugs are known to kill cancer cells by preventing cell cycle. Various secondary metabolites of some plants act by damaging the DNA during the S-phase of the cell cycle or by blocking the formation of the mitotic spindle in M-phase (Periyanyagam, Kasirajan, Karthikeyan, & Kumuda, 2013). In this present investigation, the *Theobroma cacao* seed extract was shown to inhibit prophase stage of mitotic cell division. In the *A. cepa* assay, *Theobroma cacao* seed extract was found to exhibit a dose dependent antimitotic action on *A. cepa* root meristematic cells, as indicated by inhibition of root growth (reduced root number and length) and decreased mitotic index after 24 hr of treatment. The maximum inhibition of root growth was observed at 100% concentration of the extract affecting the rootlet morphology (shrinking of the rootlets and dark brown color). There was reduction in the number of dividing cells in different phases of cell cycle. The antimitotic activity was found at maximum at the concentration of 100%, this exhibited the lowest mitotic index of 18.76% compared to the control which gave a mitotic index of 88.74%, the ethno-medicinal importance of this observation is that the extract possesses antiproliferative property, which is a potential anticancer property, however, further research on mammalian cells like bone marrow cells should be carried out to validate this observation.

5 | CONCLUSION

The present study revealed an additional ethno-medicinal importance of cocoa seeds as an antiproliferative agent with a very potent

free radical scavenging properties. *Theobroma cacao* seed extract has a high polyphenol content, and possesses antioxidant activity against free radicals. The seed extract revealed a potential antitumor activity, as shown in its effect on the mitotic cell division in *A. cepa* assay, which was significant in a concentration dependent manner. This study presents a far reaching health relevance, as *Theobroma cacao* seed extract could be projected as a functional health boosting nutritional formula, in addition to supplying essential nutrients, it also possesses preventive and therapeutic properties against the development of cancer. Further study is required to establish the antitumor activity of the seeds' isolated compounds, *in vivo* and *in vitro*, using other methods, most especially the use of human cell lines.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest concerning this study.

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