Morinda lucida (ML) and Taraxacum officinale (TO) are plants commonly used in Traditional herbal medicines in West Africa for treatment of several ailments. The ML is traditionally used to treat malaria whilst TO is used to manage hypertension. Both plants have also been claimed to have anticancer properties. In this study, we investigated the antiproliferative effects of aqueous extracts of ML and TO, including ML leaves (MLL), ML stems (MLS), TO leaves (TOL) and TO stems (TOS), in human promyelocytic leukemia (HL-60) cells. We also investigated the antioxidant activities and induction of apoptosis by the extracts. The tetrazolium-based colorimetric assay (MTT), was used to assess antiproliferative effects, whilst induction of apoptosis was determined using DNA fragmentation and cell morphological changes. Antioxidant activity via free radical scavenging was evaluated using 2,2-diphenyl-1-picryl hydrazyl (DPPH) method. Among the extracts, MLL and TOS were strongly cytotoxic to HL-60 cells, whereas MLS and TOL showed weaker effects on the cells. All extracts induced apoptosis characterized by cell morphological changes and DNA fragmentation. Furthermore, our data demonstrated that the extracts possess antioxidant activity. These results suggest that MLL and TOS partly exert antiproliferative action through apoptotic cell death in HL-60 cells. Thus, the extracts contain antiproliferative components and further studies are required for isolation and characterization anti-cancer activities of individual and combined isolated components.
INTRODUCTION
The plant *Morinda lucida* belongs to the family Rubiaceae and has anti-inflammatory, anti-pyretic, analgesic, intestinal motility, gastric emptying properties, hypoglycemic effect and antimalarial activities [1,2]. In West Africa it is one of the four plants used for treatment of fevers [3]. The leaves have been reported to possess anticancer trypanocidal, vasorelaxant, antibacterial and antifungal activities [4-6]. The methanolic stem bark extract of the plant was found to be cytotoxic with LD$_{50}$ value of 2.6 µg/ml using the brine shrimp lethality assay [7]. Olaniyan and Babatunde, reported that aqueous extract of *M. lucida* has antioxidant activity, using plasma myoglobin, transferrin, superoxide dismutase (SOD), and glutathione (GSH) peroxidase [8]. According to Adewunmi et al. [9] the major constituents of *M. lucida* are various alkaloids, anthraquinones and anthraquinols. Two known triterpenic acids, ursolic and oleic acids were isolated from the leaves [10]. Ursolic acid is well known for its strong cytotoxic and apoptotic effect [11].

The plant *Taraxacum officinale* belongs to the family Asteraceae and is used for the treatment of diseases including respiratory tract infections, hepatitis, bile and liver malfunctions [12]. Earlier studies have indicated that *T. officinale* has anticancer properties [13-15]. A previous study reported that aqueous extracts of *T. officinale* leaves decrease growth of MCF-7/AZ breast cancer cells [16]. Phytochemicals in *T. officinale* include sesquiterpene lactones, triterpenoids, tannins, alkaloids, flavonoids, steroids and phenolic acids [17]. Oleic acid has also been found in the stem of *T. officinale* [18]. Jung-Hye et al. [19] showed that taraxanic acid, a sesquiterpene present in the leaves of *Taraxacum coreanum* NAKAI, had potent anti-proliferative activity against human leukemia HL-60 cells. The flavonoid, luteolin has been found to be a potent anticancer component of *T. officinale* [14, 15]. Luteolin kills cancer cells via induction of apoptotic cell death in several cell types. *T. officinale* has also been reported to have antioxidant activities [20, 21].

Effects of aqueous extracts of *M. lucida* and *T. officinale* on human promyelocytic (leukemic) HL-60 cell line have not yet been report. Thus, in the present study we investigated the aqueous leaf and stem extracts of *M. lucida* and *T. officinale* for antiproliferative activity (a characteristic of anticancer agents) and induction of apoptosis in HL-60 cells as well as antioxidant activities. Aqueous extracts were used in this preliminary study since it is form in which Traditional Medicine Practitioners in Ghana have applied the plants components and claimed their efficacy. The plants are readily available and not expensive, and could be developed to serve as alternative therapies in the face of drug resistance.

In Ghana cancer is the fourth leading cause of death, with hematopoietic cancer accounting for a high number of deaths caused by cancer [22]. Thus, this study provides scientific information on the effect of the commonly used medicinal plants, *M. lucida* and *T. officinale* on HL-60 leukemia cells as well as antioxidant activities and mode of action.

MATERIALS AND METHODS
Materials
RPMI-1640 medium, fetal bovine serum (FBS), penicillin-streptomycin solution, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT), trypsin, ethylenediaminetetraacetic acid (EDTA) and 2,2-diphenyl-1-picryl hydrazyl (DPPH) were obtained from Sigma Chemical Company (St. Louis, MO, USA). RNase A and
dimethyl sulfoxide (DMSO) were obtained from Wako Pure Chemical Industries (Osaka, Japan) and DNA marker and loading dye were purchased from Watson (Japan). The Human promyelocytic cell line (HL-60) was obtained from the RIKEN BioResource Center Cell Bank (Japan). All other chemicals and reagents used were of analytical grade and obtained from standard suppliers. The stem and leaves of *M. lucida* (GC 45930) and *T. officinale* (GC 45929) were collected in Accra, Ghana in September 2012 and identified by a taxonomist at the University of Ghana Herbarium, Accra, where voucher specimens were deposited.

**Preparation of aqueous extracts**
The stems and leaves of *M. lucida* and *T. officinale* were air-dried and pulverized separately. Pulverized samples were extracted with distilled water at 80°C for 1 h and centrifuged at 450 rpm for 20 min at 25°C. The supernatants were collected and pellets re-suspended in distilled water to repeat the extraction procedure. The two supernatants for each sample were pooled together and freeze-dried using a LABCONCO® freeze dryer.

**Cell Culture**
The HL-60 cells were cultured in RPMI-1640 medium, supplemented with 10% FBS and 1% penicillin–streptomycin solution. Cultured cells were maintained in a humidified incubator at 37°C in the presence of 5% CO₂.

**Determination of cell viability**
The antiproliferative effects of aqueous extracts of *M. lucida* and *T. officinale* were determined using the MTT assay with slight modifications as described by Appiah-Opong et al. [23]. Cells were plated at 1x10⁴ cells/well in a 96-well plate and treated with varying concentrations of plant extracts (0 – 1.25 mg/mL). Treated cells were incubated as mentioned above for 72 h, before MTT treatment. Twenty microlitres of MTT solution (2.5 mg/mL in PBS) was added to each well and incubation was continued for 4 h. The formazan crystals formed in each well were dissolved with acidified isopropanol containing 1% Triton-X. The amount of formazan formed was determined by measuring the absorbance at 570 nm with a microplate reader (Tecan Infinite M200, Grodig, Austria). Triplicate experiments were performed.

**Induction of apoptosis**

**Observation of cell morphological changes**
Morphology of cultured HL-60 cells was examined following treatment of the cells with aqueous extracts of *M. lucida* and *T. officinale* [11]. Intact cells were considered viable whereas the presence of fragments (apoptotic bodies) was indicative of apoptosis. HL-60 cells were seeded into 6 cm petri dishes at a density of 1x10⁶ cells. Cells were treated with specific concentrations of leaf and stem extracts and incubated for 24 h at 37°C. Ursolic acid (UA) was used as positive control. Cells were then observed under a phase contrast microscope (magnification 20X) to determine the degree of cell fragmentation and blebs.

**DNA fragmentation analysis**
The DNA fragmentation and agarose gel electrophoresis were performed as described by Uto et al. [11]. The HL-60 cells (1x10⁶ cells/well) were treated with leaf and stem extracts of *M. lucida* and *T. officinale* at concentrations close to the IC₅₀ values obtained.
with the MTT assay or vehicle (negative control) for 24 h. Usorlic acid was used as positive control. The treated cells were washed in ice-cold phosphate buffer saline (PBS) and harvested by centrifugation. The pellets were re-suspended in cell lysis buffer (50 mM Tris at pH 8.0, 10 mM EDTA, 0.5% sodium dodecyl sulphate (SDS) with 0.2 mg/ml RNase A). After incubation for 30 min at 50°C, 0.1 mg/ml proteinase K was added and the cells were incubated overnight. DNA fragments were isolated and separated on 2% agarose gel, and finally visualized under UV illumination after staining with ethidium bromide.

Free radical-scavenging activity
Free radical scavenging activities of the leaf and stem extracts of *M. lucida* and *T. officinale* were determined by the DPPH method with slight modification [24]. Methanolic solution of DPPH (0.5 mM) was added to equal volumes of each extract at different concentrations in a 96-well microtitre plate. The samples were incubated at room temperature for 20 min and absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as positive control. Duplicate experiments were performed. Percent antioxidant activity of plant extract was calculated using the following formula:

\[
\text{% Antioxidant activity} = \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100,
\]

where \(A_0\) is the absorbance of the control and \(A_1\) is the absorbance of the test sample (extract). The EC\(_{50}\) value of each extract was calculated from a plot of percent antioxidant activity versus concentration of plant extract.

Statistical analysis
The percent cell viability was evaluated using the formula of Ayisi et al. [25] and plotted against extract concentrations in order to calculate the 50% inhibitory concentrations (IC\(_{50}\)). Analyses of antioxidant activities were performed using non-linear regression analysis with GraphPad Prism version 5.01 (GraphPad Prism software Inc. San Diego, CA). Data are expressed as means of two or three experiments \(\pm\) SD. Significant differences were determined using Student’s t-test. P values less than 0.05 was considered statistically significant.

RESULTS
Cell viability assay
The effect of aqueous leaf and stem extracts of *M. lucida* and *T. officinale* on HL-60 cell growth was assessed using the MTT assay. Figure 1 shows the inhibition of HL-60 cell growth after treatment with four extracts including ML leaf (MLL), ML stem (MLS), TO leaf (TOL) and TO stem (TOS) extracts, within concentration ranges of 0-1.25 mg/mL for 72 h incubation period. The results showed concentration-dependent inhibition, with IC\(_{50}\) values of 0.4, 0.171, 0.54 and 0.071 mg/mL recorded for MLL, MLS, TOL and TOS, respectively.

Induction of Apoptosis
Very few apoptotic bodies were observed in the negative control culture, while a higher percentage of apoptotic bodies were observed in the positive control (UA) and cells treated with MLS, MLL, TOS and TOL (Figure 2A). Furthermore, DNA fragmentation was examined based on a classical DNA ladder using agarose gel electrophoresis. Figure 2B shows the fragmentation of the DNA of HL-60 cells in a pattern similar to that observed.
in the positive control (UA) after treatment with aqueous extracts of ML and TO for 24 h.

**Free radical scavenging activity**
The antioxidant activities of the plant extracts were investigated by DPPH scavenging inhibition method. Figure 3 shows the free radical scavenging activity of aqueous extracts of MLL, MLS, TOL and TOS. The half maximal effective concentration (EC<sub>50</sub>) values for the extracts were in the range 0.1 to 0.35 mg/mL. TOL exhibited the strongest antioxidant activity with EC<sub>50</sub> value 0.08 mg/mL.

**DISCUSSION**
Cytotoxicity is an important measure of the anticancer activity of a therapeutic agent, whilst apoptosis is a preferred mechanism of action of such agents. Antioxidants also play a critical role in scavenging reactive substances that could otherwise cause a plethora of diseases including cancer. Apoptosis, is important mechanism for the maintenance of cellular homeostasis by regulating cell division and cell death [26]. The process is mediated by activation of certain conservative intracellular pathways resulting in the exhibition of peculiar characteristics by apoptosed cells such as morphological changes and DNA fragmentation. Several studies have shown that apoptosis is related to cancer, since cancer cells are also characterized by reduced apoptosis [27, 28]. Thus, activation of apoptotic pathways is considered an important mechanism employed by most cytotoxic drugs to destroy cancer cells [28].

In the present study, the cytotoxic effects of aqueous extracts *M. lucida* and *T. officinale* on human promelocytic HL-60 leukemia cells, the mechanism of cytotoxicity and antioxidant activities were investigated. *M. lucida* and *T. officinale* are plants commonly used in Traditional herbal medicines in West African for the treatment of several ailments as a result of their numerous therapeutic phytochemical constituents. The aqueous extracts showed significant and dose-dependent growth inhibitory effects on the HL-60 cells. This effect appeared to be mediated by the induction of apoptosis as evidenced by the morphological changes (cell shrinkage and formation of apoptotic bodies) and cellular DNA fragmentation after treatment with the aqueous plant extracts and ursolic acid. The latter has been shown to induce apoptosis *in vitro* and *in vivo* via activation of the STAT 3 pathway [29, 30]. Previous studies carried out by other researchers have shown that leaves of *M. lucida* possess ursolic acid [10]. This could partly account for the apoptosis-inducing potential shown by *M. lucida*.

The compound oleanolic acid which is known to inhibit cell proliferation and induce apoptosis has been found in the stem of *T. officinale* [18, 31]. This compound has the similar backbone structure as ursolic acid with slight differences in the substituents on carbon 20. Furthermore, taraxanic acid, a sesquiterpene present in the leaves of *T. coreanum* has also been shown to exhibit potent antiproliferative activity against human leukemia HL-60 cells [19]. Thus, the presence of oleanolic and taraxanic acid may be partly responsible for the growth inhibitory and apoptotic effects of the *T. officinale* extracts.

Reactive oxygen species (ROS) are a class of highly reactive molecules that can cause damage to cells and tissues during various infections and degenerative disorders including cancer [32]. Due to the critical role of scavenging of free radicals including ROS by antioxidants this property in medicinal plants is considered very relevant. *M. lucida* leaf extracts showed a stronger antioxidant activity than the stem extract. Earlier studies also showed similar antioxidant activity in methanolic extracts of the stem bark.
of *M. lucida* [33, 34]. The presence of flavonoids in the plant may partly account for the antioxidant activity, since flavonoids are known to possess strong antioxidant activity [35]. *T. officinale* leaf extract also showed strong antioxidant activity, which was 2.5 fold higher than that recorded for the stem extract. Thus, antioxidant properties of these plants could contribute to their anticancer property.

In conclusion, our investigation has shown that *M. lucida* and *T. officinale* extracts have antiproliferative activity and induce apoptotic activity in human promeolytic HL-60 leukemia cells. The *M. lucida* and *T. officinale* extracts demonstrated antioxidant potential that represents a protective mechanism against reactive oxygen species (ROS) associated with cancer. The stem extract of *T. officinale* had showed the strongest antiproliferative activity, while the leaf extracts gave the strongest antioxidant activity. Our results lend support to earlier reports on the anticancer and antioxidant activities of the plants. These findings warrant further studies on the development of the plant extracts as alternative anticancer therapies.

**Figure 1.** Effects of MLL, MLS, TOL and TOS on HL-60 cell viability

Antiproliferative effect of aqueous extracts of MLS and MLL (A) and TOS and TOL (B) on HL-60 cells. The data represents the mean ± S.D of two experiments as described in the Methods section.
Figure 2. Induction of apoptosis in HL-60 cells by MLL, MLS, TOL and TOS (A) HL-60 cells were treated with the indicated concentrations of aqueous extracts of ML and TO and positive control (ursolic acid, UA) for 24 h. Cell morphology was
observed with a camera-aided phase contrast microscope (magnification 20×); (B) DNA fragmentation by aqueous extracts of ML and TO. HL-60 cells were treated with the extracts for 24 h and the fragmented DNA was analyzed by agarose gel electrophoresis. M is the 100-bp DNA marker.

Figure 3. The free radical scavenging activities of aqueous extracts *T. officinale* leaves (A), *T. officinale* stems (B), *M. lucida* leaves (C) and *M. lucida* stems (D) were determined as a measure of total antioxidant potential.

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