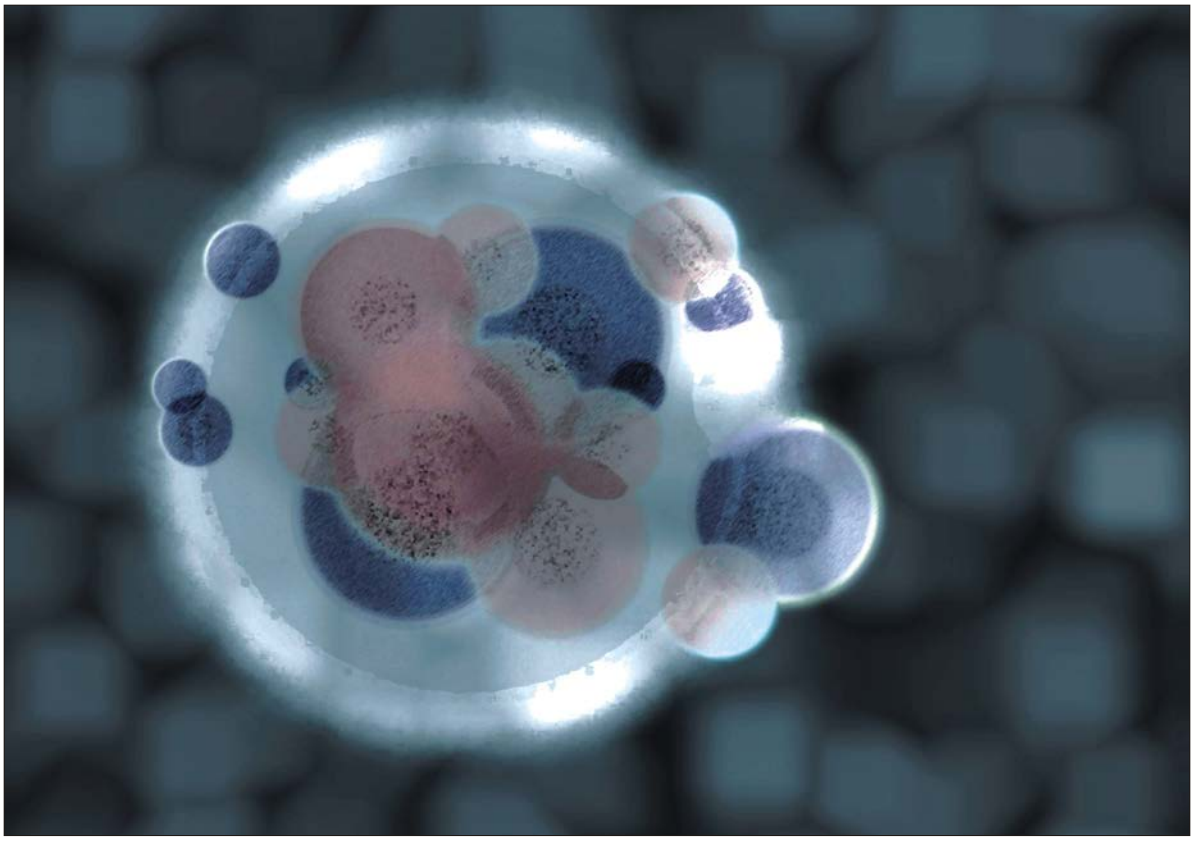

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Research Article
Effects of Aqueous Extracts of *Taraxacum officinale*, *Morinda citrifolia*, *Milletia thonningii* and Noni on Mitochondrial Function

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Abstract

Background and Objectives: Mitochondria are commonly referred to as the cellular "powerhouses" because they produce 90% of the energy necessary for cell survival in the form of adenosine triphosphate by oxidative phosphorylation. When xenobiotics adversely affect the oxidative phosphorylation pathway, it results in xenobiotic-induced bioenergetic failure leading to mitochondrial failure and/or dysfunction, which have been implicated in host of diseases such as neurodegenerative conditions. Conversely, a positive interaction between xenobiotics and the oxidative phosphorylation machinery results in positive drug outcomes. The present study investigated the effects of aqueous extracts of the leaves of 3 common medicinal plants *Taraxacum officinale* (TO), *Morinda citrifolia* (MC), *Milletia thonningii* (MT) and a locally prepared Noni (N) on mitochondrial function. **Materials and Methods:** A substrate-inhibitor titration was used to assess the effects of the extracts on mitochondrial function in permeabilised cardiac muscle. The cytotoxic effects of the extracts on Jurkat leukemia cells and MCF7 breast cancer cells were assessed using the MTT assay. The antioxidant activities were analysed using the DPPH scavenging assay, phenolic contents and basic phytochemical components were also analyzed by employing standard protocols. **Results:** The extracts showed varying phytochemical constituents and antioxidant activities with the Noni showing the lowest phenolics content but the highest antioxidant activity. None of the extracts significantly alter mitochondrial function directly *in situ* or indirectly in the cancer cell-lines. **Conclusion:** The plant extracts and the locally prepared Noni exhibited different photochemical compositions and antioxidant activities and caused different in-significant and non-adverse effects on mitochondrial function.

Key words: Mitochondrial function, plant extracts, antioxidant activities, phytochemical compositions

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mitochondria are commonly referred to as the cellular "powerhouses" because they produce 90% of the energy necessary for cell survival in the form of adenosine triphosphate (ATP)[1]. They also perform other important functions including redox signalling[2], calcium homeostasis[3] and play a crucial mediating role in programmed cell death (apoptosis)[4]. Additionally, mitochondria have unique properties such as presence of own DNA[5], cardiolipin-rich inner membrane and negatively charged (alkaline) interior[6], which provide a number of primary and secondary targets for drugs[7].

The production of ATP by the mitochondrion is via the process of oxidative phosphorylation (OXPHOS), which couples oxidation of reduced substrate to phosphorylation of ADP to ATP[8]. OXPHOS involves five enzyme complexes (complexes 1-5). Complexes 1-4 constitute the respiratory chain or the electron transport chain (ETC) and complex 5 is the ATPase[8]. The OXPHOS pathway is known to be sensitive to many compounds including respiratory chain substrates and inhibitors, uncouplers and ATPase inhibitors. The pathway is therefore largely under the influence of many xenobiotics such that over 2,000 drugs in clinical use have been identified to interfere with energy metabolism[9]. This influence of xenobiotics on the OXPHOS pathway results in xenobiotic-induced bioenergetic failure leading to mitochondrial failure and/or dysfunction. Mitochondrial failure and/or dysfunction is implicated in host of diseases such as neurodegenerative conditions (Amyotrophic lateral sclerosis, Alzheimer's, Parkinson's Disease), epilepsy, autism, diseases of the cardiovascular system, liver, kidney, cancer and diabetes[10]. Thus, since medications for many diseases cause unwanted toxicity to the mitochondria, it is extremely critical for drug discovery and development researchers to be able to predict and prevent the serious side effects of these compounds and/or preparations.

However, though many plant bioactive compounds including polyphenols have been reported to have beneficial effects on mitochondrial function[11], data on the effect of Ghana's medicinal plant extracts on mitochondrial function are scarce. *Phyllantus urinaria*, for instance, has been found to inhibit complex 1 and complex 2 of the ETC and significantly induces a decline in mitochondrial respiration[12]. The dichloromethane extract of the seeds of *Millettia thonningii* has been shown to inhibit complex 1 activity and this inhibition is believed to account for its molluscicidal and schistosomicidal activities[13]. Similarly, the ethanolic extract of *Paulinia pinnata* has been found to inhibit complex II activity, which could account for its reported toxicity in fish[14].

The leaves of *Taraxacum officinale* (TO) are popular among Ghanaians as a vegetable and/or beverage. In addition, extracts of the leaves and various parts of *T. officinale*, *Morinda citrofolia* (MC) and *Millettia thonningii* (MT) are present in common herbal remedies, widely available without prescription in Ghana. Noni fruit juice (N), prepared by fermentation of fruits from *Morinda citrifolia* has become popular in recent times in Ghana. It is used as a food supplement for the enhancement of general well-being and as an energy booster. Therefore the present study investigated the effects of a locally prepared Noni fruit juice (N) on mitochondrial function *in situ* compared with aqueous extracts of the leaves of the three common medicinal plants, *Taraxacum officinale* (TO), *Morinda citrifolia* (MC), *Milletia thonningii* (MT). The study also investigated the cytotoxic effects of the extracts on Jurkat leukemia cells and MCF7 breast cancer cells using the MTT assay as an indirect measure of their effects on mitochondrial function. The antioxidant activities, phenolic contents and basic phytochemical components were also analyzed.

MATERIALS AND METHODS

Collection of plant materials: The leaves of the three medicinal plants were harvested at different locations. *Taraxacum officinale* Weber leaves were harvested at Adabraka whilst *Morinda citrifolia* Linn leaves were harvested at Ayikuma. *Milletia thonningii* (Schum. and Thonn.) Baker leaves were harvested near the Department of Botany, University of Ghana. All the plant materials were authenticated at the Plant Development Department of the Centre for Plant Medicine Research, Mampong-Akuapem. The Noni was purchased from the Royal Noni Factory at Dzorwulu, Accra, Ghana.

Preparation of aqueous plant extracts: The plant materials were air-dried away from direct sunshine for 3 weeks and then pulverized with a blender. Aqueous extracts were prepared from the pulverized samples by adding 500 mL of distilled water to 50 g of the pulverized sample (10% w/v). The resulting mixture was heated at 80°C for 1 h, cooled to room temperature and centrifuged at 4500 g for 20 min. The supernatant was decanted into a clean tube. The pellet was re-suspended in 500 mL distilled water. The heating and centrifugation of the extracts were repeated and the supernatant obtained was again decanted, collected and added to the previous one. The plant extracts and the NFJ were freeze-dried and stored at 4°C.

Preliminary phytochemical screening: The crude extracts and the Noni juice were screened for the presence of 6 phytochemicals: alkaloids, cardiac glycosides, flavonoids, saponins, tannins and terpenoids.

The presence of alkaloids was assayed as previously described[15] with slight modification. An amount of 0.1 g of each of the crude extracts was weighed into separate test tubes containing 5 cm^3 of 2 M HCl solution. The solution was stirred, warmed and filtered. The filtrate from each extract was then divided into 3 equal volumes. Dragendorff's reagent was added to one portion, Meyer's reagent to the other and Wagner's reagent to the third. The presence of a yellowish or reddish brown precipitate indicated the presence of alkaloids.

The presence of flavonoids was investigated by employing the method described previously[16]. An amount of 0.1 g of each of the crude extracts was added to 5 mL of 80% ethanol. The resulting solution was filtered and divided into two. To one portion of the filtrate, magnesium turnings were added. This was followed by the addition of 0.5 mL of concentrated hydrochloric acid. The solution was monitored for 10 min to observe any colour change. Concentrated hydrochloric acid (5 mL) was added to the other portion and the solution was warmed for 5 min. The observation of a light pink colour was an indication of the presence of flavonoids.

Tests for the presence of cardiac glycosides, saponins, tannins and terpenoids were carried out as previously described[17]. An aliquot of 5 mL of each of the extract was mixed with 2 mL of glacial CH_3COOH containing 1 drop of $FeCl_3$. Concentrated H_2SO_4 (1 mL) was carefully added to the above mixture in a test tube such that the concentrated H_2SO_4 was underneath the mixture. The appearance of a brown ring indicated the presence of the cardiac glycoside constituent.

To test for the presence of saponins, 0.5 g of each extract was boiled together with 5 mL of distilled water in a water bath and then filtered. An aliquot of 2.5 mL of the filtrate was mixed with 1.25 mL of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. Three drops of olive oil was added to the froth. The formation of an emulsion indicated the presence of saponins.

The presence of tannins was tested by boiling 0.25 g of each extract in 10 mL of distilled water and then filtered. This was followed by the addition of 3 drops of 0.2 % $FeCl_3$ to the filtrate. The observation of a brownish green or a blue-black colouration indicated the presence of tannins.

An aliquot of 5 mL of aqueous extract of each plant sample was mixed with 2 mL of $CHCl_3$ in a test tube. To the aqueous extracts, 3 mL of concentrated H_2SO_4 was carefully added to the mixture to form a layer. The presence of terpenoids was indicated by an interface with a reddish brown coloration.

Assessment of total phenolics: Total phenolic content of the extracts and the Noni was determined using the Folin-Ciocalteau assay[18]. Briefly, a concentration of 5 mg mL^{-1} of each extract was prepared. In a 96-well plate, 10 µL of each extract was added to 790 µL of distilled water in each well. To this, 50 µL of Folin-Ciocalteau reagent was added. The resulting solution was thoroughly mixed and incubated in the dark for 8 min. This was followed by the addition of 150 µL of 7% Na_2CO_3 and further incubation for 2 h in the dark at room temperature. The experiment was performed in duplicates. The absorbance was read at a wavelength of 750 nm using a microplate reader (Tecan Infinite M200, Austria). Gallic acid was used as the standard phenolic compound. A gallic acid calibration curve was plotted and used to determine the phenolic content of the extracts, which was expressed as equivalent of the phenolic content of 1 g gallic acid.

Assessment of antioxidant activities of the extracts: The free radical scavenging activities of the extracts and the Noni were assessed using the 2,2 - diphenyl-1-picrylhydrazyl (DPPH) method[19]. Varying concentrations of the extracts ranging from 0-5 mg mL^{-1} were prepared from 10 mg mL^{-1} stock solution in distilled water. An amount of 10 mM butylated hydroxytoluene (BHT) was used as the positive control. The reaction mixture was made up of 100 µL of plant extract or BHT and 100 µL of 0.05 mM DPPH in 96-well plates. The experiments were performed in triplicates. The samples were incubated in the dark at room temperature for 20 min. The absorbance was then read at 517 nm using a microplate reader (Tecan Infinite M200, Austria). The radical scavenging capacity of each extract was calculated as the percent DPPH radical scavenging effect as shown below:

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of the test experiment. The EC_{50} (substrate concentration to produce 50% reduction of the DPPH) values were determined from a plot of percentage DPPH scavenging effect versus log concentration of test sample.

Assessment of the effects of the extracts on cell viability: The effect of the extracts on the viability of MCF7 breast cancer and Jurkat leukemia cell lines were assessed using the tetrazolium based colorimetric [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] (MTT) assay[20]. The MCF7 cells were maintained in DMEM supplemented with 10% FBS while the JURKAT cells were maintained in RPMI 1640 supplemented with 10% FBS. The cells were incubated at 37°C in the presence of 5% CO_2. They were then seeded (1×10^5 cells mL^{-1}) into 96-well plates and pre-treated with varying concentrations (0 -1000 μg mL^{-1}) of the extracts. Curcumin was used as the positive control and distilled water was used as the negative control. The experiment was performed in triplicates.

After incubating the cells with the extracts for 72 h, 20 μL of 2.5 mg mL^{-1} MTT solution was added to each well and incubated for a further 4 h. The reaction was then stopped with 150 μL acidified isopropanol after which the plate was incubated in the dark at room temperature overnight. Optical density was read using Tecan Infinite M200 Pro (JICA, Japan) plate reader at a wavelength of 570 nm. Percent cell survival was evaluated and IC_{50} values were determined.

Assessment of the effects of the extracts on mitochondrial respiration

Preparation of permeabilised fibres: Permeabilised mice cardiac fibres were prepared as described previously[14]. Male ICR mice between the ages of 3-5 months with an average weight of 28.75 g were obtained from the Centre for Plant Medicine Research (CPMR), Mampong-Akuapem. The animals were housed under controlled conditions for temperature, humidity and light, in accordance with international guidelines, with water and food availability *ad libitum*.

The animals were euthanised with chloroform followed by dislocation of the neck. The heart was removed immediately following cervical dislocation and placed in ice-cold biopsy preservation solution (2.77 mM CaK_2EGTA, 7.23 mM K2EGTA, 20 mM imidazole, 20 mM taurine, 50 mM MES, 3 mM K_2HPO_4, 6.5 mM $MgCl_2.6H_2O$, 5.7 mM ATP, 15 mM phosphocreatine and 0.5 mM DTT, pH 7.1). Fat and connective tissues were carefully removed and the cardiac tissue isolated into muscle strips. With the aid of sharp, non-magnetic forceps, the strips, immersed in ice-cold isolation solution were dissected into thin muscle-fibre bundles under a dissecting microscope. The fibre bundles were transferred to a vial containing 1.7 ml ice-cold isolation solution and 20 μL of 5 mg mL^{-1} saponin and mixed gently at 4°C for 20 min. The fibres were transferred to ice-cold respiration medium (0.5 mM EGTA, 3 mM $MgCl_2.6H_2O$, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose and 1 g L^{-1} BSA (fatty acid free), pH 7.1) and mixed gently at 4°C for 5 min. The washing step, in ice-cold respiration medium was repeated 3 times.

Measurement of mitochondrial oxygen consumption: The mitochondrial oxygen consumption was measured in presence and absence of the plant extracts using a Clark-type oxygen electrode (782 oxygen electrode system from Strathkelvin Instruments, UK). The permeabilised tissue fibres (2.5-3.0 mg wet weight) were pre-incubated for 2 min with 2 μL of 10 mg mL^{-1} of the extracts. Equal volume of the water was used as control. A substrate-inhibitor titration was then used as described previously[14], to elucidate respiratory chain function. Briefly 2 mM Malate and 10 mM Glutamate (G/M) were added to stimulate State 2 respiration via complex 1 before 5 mM ADP was added to activate oxidative phosphorylation (State 3 respiration). Next, 1 μM Rotenone (Rot) was added to inhibit complex 1, before 10 mM Succinate (Succ) was used to stimulate respiration via complex 2. Next, 2.5 μM Antimycin A was used to inhibit complex 3 and thus all respiration supported by substrates for complexes 1 and 2. Finally 2 mM Ascorbate (Asc) and 0.5 mM N,N,N′,N′-tetramethyl-p-phenylenediamine dihydrochloride (TMPD) (Asc/TMPD) were added to support respiration via complex 5 before 10 μM Cytochrome c was added to assess outer mitochondrial membrane intactness. Oxygen consumption was allowed to reach steady state following each addition and the rate of respiration was analysed using the 782 system software (Strathkelvin Instruments). Respiration rates were presented as oxygen consumption per mg wet weight of tissue and the respiratory control ratio (RCR) calculated as State 3/State 2 as a measure of mitochondrial coupling.

Statistical analysis: Comparisons between means were performed and significance was evaluated by one-factor analysis of variance using Microsoft Excel. Probability value of $p<0.05$ was used as the criteria for significant differences.

RESULTS

Total phenolics: The phenolic content of the plant extracts and the Noni expressed as equivalent of 1 g gallic acid were as indicated in Fig. 1. The extract from *Milletia thonningii* (MT) gave the highest phenolic content of 0.24±0.00 g GAE. The phenolic contents of the extracts from *Morinda citrifolia* (MC) and *Taraxacum officinale* (TO) were found to be similar

Table 1: Phytochemical composition of the plant extract and Noni

	Extract			
Phytochemicals	*Taraxacum officinale* (TO)	*Morinda citrifolia* (MC)	Noni	*Millettia thonningii* (MT)
Alkaloids	-	+	-	-
Flavonoids	+	+	-	-
Glycosides	-	-	-	-
Saponins	+	+	+	+
Tannins	+	+	-	+
Terpenoids	-	-	-	-

(0.07±0.00 g GAE) whilst the Noni had the lowest phenolic content of 0.03±0.00 g GAE.

Phytochemical analysis: Table 1 shows the results from the preliminary phytochemical screening. The plant extracts including the Noni exhibited varying phytochemical compositions. Saponins were found to be present in all the extracts including the Noni and tannins were observed in all the extracts excluding the Noni. Alkaloids were observed in only the extract from MC and flavonoids were present in the extracts from TO and MC but absent in MT and the Noni. Neither glycosides nor terpenoids were detected in any of the extracts and the Noni.

Antioxidant activity assessment: The results of the DPPH scavenging activities of the extracts and the Noni compared with the standard (BHT) were as presented in Table 2. The extract from MC and the Noni exhibited strong DPPH scavenging properties with EC_{50} of 0.60 and 1.98 mg mL^{-1}, respectively compared to EC_{50} value of 0.43 mg mL^{-1} for the standard (BHT). The extract from TO exhibited moderate DPPH scavenging activity with EC_{50} value of 0.31 mg mL^{-1} whilst that of MT exhibited the weakest scavenging activity with EC_{50} value of 0.21 mg mL^{-1}.

Cytotoxic effect on cancer cell-lines: The results of the effects of the plant extracts and the Noni on Jurkat leukemia and MCF7 breast cancer cell lines using the MTT assay were as shown in Fig. 2 and 3, respectively. All the extracts including Noni moderately inhibited the Jurkat leukemia cells (Fig. 2b). However, neither the Noni nor the extracts inhibited MCF7 breast cancer cells (Fig. 3b). The extracts showed dose dependent cytotoxic effects on the Jurkat leukemia cells (Fig. 2b) with IC_{50} values greater than 100 µg mL^{-1} and no effect on MCF7 breast cancer cell lines (Fig. 3b). Curcumin, the positive control, inhibited both the Jurkat leukemia and MCF7 breast cancer cells with IC_{50} values of 11.4 µM (Fig. 2a) and 74.24 µM (Fig. 3a), respectively.

Table 2: Antioxidant activities of the extracts and Noni compared with BHT

Extracts	EC_{50}
BHT	0.43
Noni	1.98
Morinda citrifolia (MC)	0.60
Taraxacum officinale (TO)	0.31
Millettia thonningii (MT)	0.21

Values are Mean±SEM, n = 3

Table 3: Effects of the extracts and Noni on respiratory control ratio (RCR)

Plant extract/solvent	RCR
Solvent	2.8±0.1
Noni	2.7±0.1
Morinda citrifolia (MC)	3.2±0.2
Taraxacum officinale (TO)	3.2±0.4
Millettia thonningii (MT)	3.5±0.4

Values are Mean±SEM, n = 5

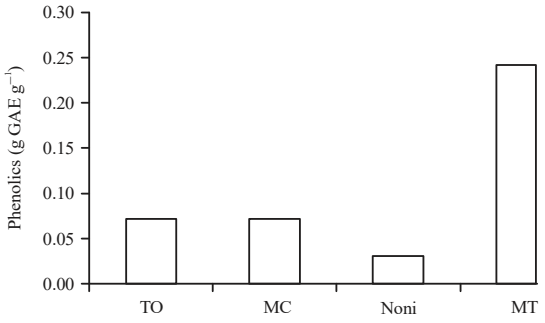

Fig. 1: Phenolic contents of the extracts and Noni

Values are Mean±SEM, n = 3, TO: Taraxacum officinale, MT: *Millettia thonningii*, MC: *Morinda citrifolia*

Effects on mitochondrial function *in situ*: The effects of the extracts and Noni on mitochondrial respiratory chain activity *in situ* were as shown in Fig. 4 and Table 3. Fig. 3 shows the effects of the extracts (MC, MT and TO) and the Noni on individual mitochondrial respiratory chain complexes whilst Table 3 shows the effect on the respiratory control ratio (RCR = sate 3/state 2 ratio), which is a measure of mitochondrial integrity.

As expected, addition of the solvent (water) to the tissue preparation did not result in oxygen consumption (basal oxygen consumption). Expectedly, all the extracts

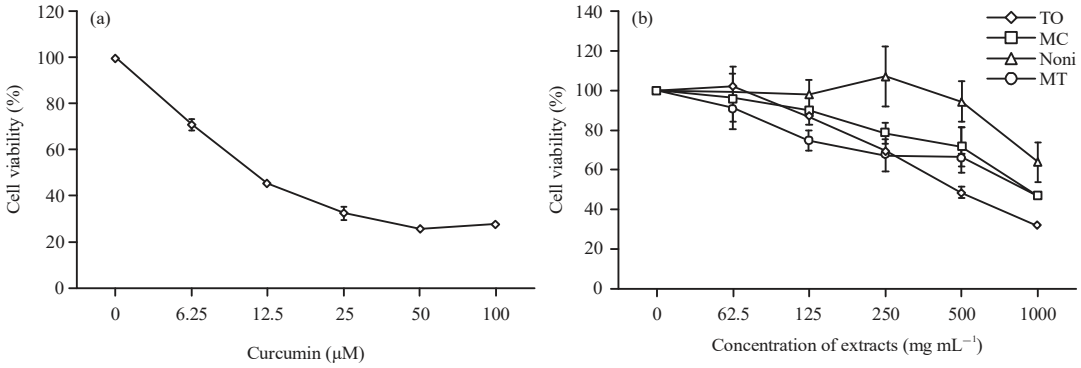

Fig. 2(a-b): Effects on viability Jurkat leukemia cells (a) Curcumin (positive control) and (b) Extracts and Noni
Data represent Mean±SEM, n = 3

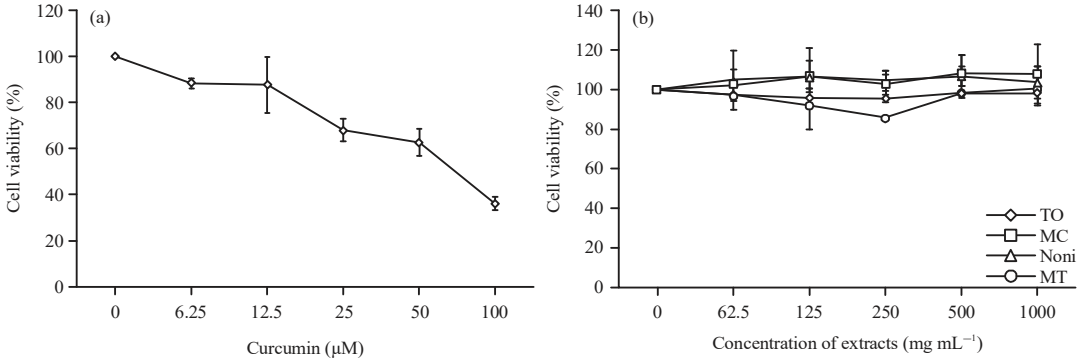

Fig. 3(a-b): Effects on viability MCF7 breast cancer cells (a) Curcumin (positive control) and (b) Extracts and Noni
Data represent Mean±SEM, n = 3

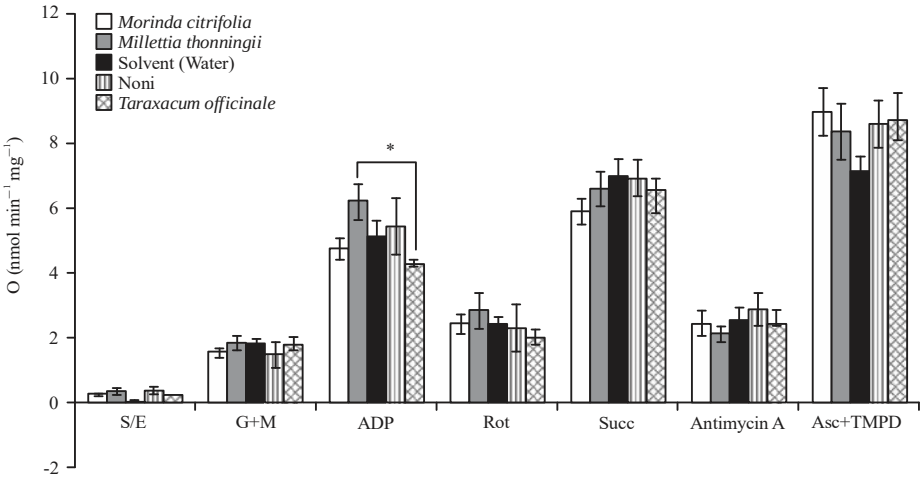

Fig. 4: Effects of extracts and Noni on mitochondrial respiratory chain activity in permeabilised mouse heart fibers
Values are Mean±SEM of 5 independent analysis, *significantly different

and Noni caused minimal but significant increase in the basal mitochondrial oxygen consumption compared to the solvent (1st set of bars) ($p \leq 0.02$). This increase in the basal respiration was however not different among the extracts.

Addition of glutamate and malate (G+M) to stimulate complex 1 (2nd set of bars) caused a significant increase in oxygen consumption (State 2 respiration) as compared to the basal respiration and this was not significantly different in the

presence of either the Noni or the extracts. On addition of ADP to stimulate State 3 respiration through complex 1 (3rd set of bars), the extract from MT caused a significant increase in the mitochondrial oxygen consumption compared to only the extract from TO but not compared to the solvent or the other extracts (MC and Noni). Addition of rotenone to inhibit complex 1 caused a remarkable decrease in oxygen consumption (4th set of bars), which was not significantly altered by any of the extracts or the Noni.

Succinate (succ) stimulated respiration through complex 2 (5th set of bars) and this was not altered by any of the extracts or the Noni, although the extract from MC caused a slight reduction in oxygen consumption. Antimycin A, which is a specific inhibitor of complex 3 reduced the succinate-stimulated respiration to the state 2 level (6th set of bars) and this was not significantly altered by any of the extracts or the Noni.

The addition of ascorbate and TMPD (Asc+TMPD), an artificial electron donor to complex 4, expectedly produced a remarkable increase in the Antimycin A inhibited respiration (7th set of bars). This increase in respiration was non-significantly higher in the presence of all the extracts and the Noni compared to the solvent.

Regarding the RCR, in exception of Noni, which did not alter RCR compared to the solvent, all the extracts produced a slight and non-significant increase in RCR (Table 3).

DISCUSSION

Xenobiotics including natural products can alter the mitochondrial OXPHOS pathway resulting in xenobiotic-induced bioenergetic failure leading to mitochondrial failure and/or dysfunction. The present study therefore investigated the effects of aqueous extracts of the leaves of three common medicinal plants *Taraxacum officinale* (TO), *Morinda citrifolia* (MC), *Milletia thonningii* (MT) and a locally prepared Noni on mitochondrial function. The cytotoxic effects of the extracts on Jurkat leukemia cells and MCF7 breast cancer cells were also analyzed using the MTT assay as an indirect measure of their effects on mitochondrial function. In addition, the antioxidant activities, phenolic contents and basic phytochemical components were also analysed.

Phytochemical constituents and antioxidant activities: Extracts from herbs, spices and medicinal plants contain variety of phytocompounds/phytochemicals, which can be broadly classified as alkaloids, terpenoids, phenolics and flavonoids[11,21]. These phytochemicals often determine the physiological action of the medicinal plant extracts on the human body and are known to be responsible for their medicinal properties and health benefits[22]. The phytochemical screening revealed that the extracts possess varying phytochemical compositions (Table 2). The extract from MC contained a wider variety of phytochemicals (alkaloids, flavonoids, saponins and tannins) and the Noni was positive for only saponins. Additionally, the extracts and the Noni exhibited varying levels of total phenolics. The highest phenolics content was measured in the MT extract and the lowest in the Noni.

In addition to environmental factors contributing to variation in phytochemical composition of plant extracts, phytochemical constituents of plants also vary among species and genera[21]. Phenolics, tannins and flavonoids have been reported in the aqueous extract of *Taraxacum officinale*[23]. *Morinda citrifolia* leaves extract were reported to contain flavonoids, alkaloids, saponins, tannins and steroids[24]. *Milletia thonningii* leaves extract is known to contain flavonoids, alkaloids, saponins, tannins, steroids, terpenes and glycosides[25] and fatty acids, glycosides and iridoids have been observed in Noni[26].

Consequently, the extracts exhibited different levels of antioxidant activity. However, no relationship was observed between the types of phytochemical constituents, phenolics content and antioxidant activity. Noni, which tested positive for only saponins gave the highest antioxidant activity. Saponins and polyphenols including flavonoids constitute phytochemicals with known antioxidant properties[11,27]. The extract from MC produced significantly lower antioxidant activity compared to the Noni although it tested positive for the highest variety of phytochemicals. MT, which had the highest phenolics content, gave moderate and weak antioxidant activity. These observations suggest that the differences in antioxidant activities are not due to the quality of phytochemical composition.

The Noni is a purported health drink prepared from the fruits of *Morinda citrifolia*. The global popularity of Noni has increased dramatically in the past decade[28] and in Ghana, both the imported and local varieties are sold either in the open market or pharmacies across the country. The significantly high antioxidant activity exhibited by the Noni may account for its wide use as a food supplement for the promotion of general well-being.

Cytotoxic activity of the extracts and the Noni: The succinate dehydrogenase system of the mitochondrion converts MTT to formazan in viable cells[29]. Therefore, the cytotoxicity of the selected plant extracts was determined as an indirect measure of the effects of the extracts on mitochondrial function using

the MTT assay. According to the US National Cancer Institute, a plant extract is considered to have active cytotoxic effect if the IC_{50} value is 30 μg mL^{-1} or less[30,31]. Thus, the extracts and the Noni exhibited weak or no cytotoxic properties against the 2 cell lines (Jurkat leukemia and MCF7 breast cancer cells) suggesting that they possibly lack the potential to significantly alter mitochondrial respiratory chain activity especially at complex 2. It also demonstrates the fact that these extracts are generally safe.

Effects of the extracts and Noni on mitochondrial function *in situ*: Analysis of the mitochondrial oxygen consumption using the Clark-type oxygen electrode was used as a direct measure for the effects of the extracts on mitochondrial function. As shown in Fig. 4, apart from MC, which significantly increased complex 4 activity, none of the extracts significantly influenced mitochondrial respiratory complexes activity. Additionally, in exception of MT, which significantly increased the complex 1-stimulated state 3 respiration compared to TO, the effects of the extracts on the mitochondrial respiratory chain activity were generally similar. Thus, the results from the mitochondrial oxygen consumption measurements confirmed the indication from the cell viability assay that the extracts did not alter mitochondrial function especially at complex 2.

Although studies on crude medicinal plant extracts and mitochondrial function are scanty Ocloo *et al.*[14], reported that whilst the ethanolic fraction of *T. officinale* (TO) leaves significantly increased the respiration rate in presence of rotenone, the aqueous fraction did not. Thus, the present results confirmed the previous finding and suggest that phytocompounds responsible for the stimulatory effect of TO extract on rotenone inhibited respiration are contained in the ethanolic fraction. While, crude extract and isolated compounds from the seeds of *M. thonningii* have previously been reported to have inhibitory effect on mitochondrial complex 1 activity[13,32], there has been no report on the leave extracts. The potential health benefits of Noni have been well documented[33], Meanwhile, Noni is claimed to have an energy boosting effect suggesting that it may have an effect on cellular energy metabolism and for that matter mitochondrial function.

Ascorbate/TMPD is an artificial electron donor to complex 4 and any compound that improves electron donation by ascorbate/TMPD will increase complex 4 activity. Strong antioxidants have been reported to possess such properties. It is therefore not surprising that ascorbate/TMPD stimulated respiration was higher in presence of all the plants extracts and the Noni. However, the effect of the extracts and the Noni on ascorbate/TMPD stimulated respiration do not appear to be dependent on their antioxidant capacity with the Noni producing the same effect as the aqueous extract of MT although the Noni exhibited about ten-fold higher antioxidant activity (Table 3).

The mitochondrial RCR is the ratio of state 3 to 4 respiration[34]. However, in experimental setup such as *in situ* study, where state 4 respiration is not practically attainable, RCR is roughly estimated as the ratio of state 3 to state 2 respirations[34]. It is an indirect measure of how tightly or loosely the mitochondrial substrate oxidation is coupled to ATP synthesis[34]. That is, how much of the mitochondrial oxygen consumption results in ATP synthesis. A higher RCR is a measure of tightly coupled mitochondria and vice versa[34]. In the present study, the RCR, in exception of Noni, which did not alter RCR compared to the solvent, RCR was slightly higher in presence MT, MC and TO compared to the solvent. This is consistent with the observation by Ocloo *et al.*[14], demonstrating high RCR in presence of extracts from selected medicinal plants including *Taraxacum officinale*. Intriguingly, although Ocloo *et al.*[14] associated their observation with antioxidant capacity of the plant extracts[14], Noni, which exhibited the highest antioxidant activity in the present study, did not alter the RCR.

CONCLUSION

In conclusion, the present finding shows that the aqueous extracts from the leaves of *Taraxacum officinale* (TO), *Morinda citrifolia* (MC), *Milletia thonningii* (MT) and a locally prepared Noni exhibited different photochemical compositions and antioxidant activities and produced different non-significant and non-adverse effects on mitochondrial function. The extracts also did not have significant cytotoxic effect and are thus generally safe.

SIGNIFICANCE STATEMENT

This study gives insight into the potential effects of the extracts of selected medicinal plants on mitochondrial function. Mitochondria are the cellular powerhouses and any adverse effect on their function by medicinal plant extracts could lead to what is termed xenobiotic-induced bioenergetics failure. The study revealed that none of the plant extracts adversely affected mitochondrial function either directly or indirectly. This study has contributed to knowledge on the effects of medicinal plant extracts from Ghana on mitochondrial function, which is largely lacking. The study has

also provided a background data on Ghana's medicinal plant extracts in the field of mitochondrial pharmaco toxicological research.

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