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Research Article

Solvent Fractions of *Daniellia oliveri* ((Rolfe) Stem Bark Modulate Rat Liver Mitochondrial Permeability Transition Pore

Achem J, *Oyebode O.T, Akinwole M, Bolarin O, Malgwi J.M and Olorunsogo O.O
Laboratories for Biomembrane Research and Biotechnology, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.

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Abstract
Mitochondrial-dependent cell death is initiated by the release of cytochrome c due to the opening of the mitochondrial permeability transition (mPT) pore, a pharmacological target for drug development in the treatment of diseases associated with dysregulated apoptosis. *Daniellia oliveri* (DO) is traditionally used in management of breast tumours, sickle cell anaemia and diabetes. This study evaluated the effects of solvent fractions of *D. oliveri* stem bark extract on the modulation of the mPT pore. The crude ethanol extract (EEDO) of the stem bark was partitioned successively with n – hexane (HFDO), chloroform (CFDO), ethyl acetate (EAFDO) and ethanol (EFDO) to obtain their respective fractions. Mitochondrial permeability transition (mPT), mitochondrial ATPase (mATPase) activity, cytochrome c release and mitochondrial lipid peroxidation were assessed spectrophotometrically. The EEDO and EFDO significantly induced ($p < 0.05$) mPT pore opening with induction folds of 15.4 and 12.2 folds, respectively at the highest concentration (420 µg/ml) in the absence of calcium while inductive effects of CFDO and EAFDO were not significant. The inductive effect of EFDO resulted in significant release of cytochrome c to the cytosol. In contrast, all fractions of DO had mild inhibitory effects on calcium-induced opening of the mPT pore except CFDO that displayed strong reversal of pore opening (86%) greater than spermine (73%), a standard inhibitor. Furthermore, at pH 7.4, all solvent fractions of *D. oliveri* stem bark significantly ($p < 0.05$) inhibited Fe^{2+} - induced lipid peroxidation in a concentration-dependent manner and EFDO showed maximum enhancement effect of mATPase activity compared to control and other fractions. In this regard, enhancement effect of 63% was obtained at 420 µg/ml of EFDO which was comparable to 2, 4-dinitrophenol (68%), the standard uncoupler of oxidative phosphorylation These findings show clearly that *Daniellia oliveri* contains bioactive agents that either induce or inhibit calcium-induced opening of the mPT pore and therefore modulate mitochondrial-mediated cell death and this justifies the use of the plant in management of diseases associated with dysregulated cell death. Further studies on the isolation and characterization of the compounds responsible for modulating the mitochondrial permeability transition pore will therefore yield positive results in drug design in situations or diseases associated with dysregulated cell death

Key Words: *Daniellia oliveri*, Cytochrome c, Mitochondria, Apoptosis.

INTRODUCTION

Mitochondria are intracellular organelles which function in cellular energy production and various forms of cell death such as apoptosis, necrosis and mitotic catastrophy initiated by mitochondrial outer membrane permeabilization (Elmore, 2007; Osellame, 2012). The intrinsic or mitochondrial mediated pathway of cell death has been characterized and it has been shown to occur when the mitochondrial permeability transition (mPT) pore opens in response to various stimuli, thereby bringing about the release of cytochrome c and several other apoptogenic or pro-apoptotic proteins (Kroemer et al., 2005). These stimuli include high level of cytoplasmic calcium concentration, reactive oxygen species, activation of pro-apoptotic Bcl-2 family of proteins and UV damage (Webster, 2012).The cascade of events that takes place following the release of the apoptogenic proteins results in the activation of initiation and executioner caspases and the final digestion of the cell. Consequently, opening of the mPT pore and the subsequent release of cytochrome c is thought to be a 'point of no return' for mitochondrial-mediated cell death to take place (Gogvadze *et al.*, 2000). Interestingly, the mPT pore and mitochondrial-mediated apoptosis have been implicated in the pathology of several human diseases (Bernadi *et al.*, 2015). It is in this connection that the opening of the pore has become a major therapeutic target in drug development in diseases where apoptosis is dysregulated (Suh *et al.*, 2013). Despite accumulating evidence for the molecular constituents of the mPT pore, the exact nature of the proteins responsible for pore formation is under debate. This fact

**Author for Correspondence:* Tel: +234 8059638379 E-mail: ot.oyebode@mail.ui.edu.ng

notwithstanding, the number of bioactive agents that inhibit or induce mPT pore opening is on the increase (Martins, 2006; Millimouno *et al.,* 2014), and a good number is undergoing clinical trials. We have also demonstrated that bioactive agents in certain fractions of a number of medicinal plants have the potency to modulate the mPT pore (Odewusi *et al.,* 2010; Adisa *et al.,* 2013; Oyebode *et al.,* 2018,).

Daniellia oliveri (caesalpiniacea) is a plant found in the Amazon region and other parts of South America and Africa (Gentry, 1973). It is commonly known as African *copaiba* balsam or Ilorin balsam (Adaku and Okewesili, 2000). In Nigeria, it is called "iya" in Yoruba, "maje" in Hausa "ubakwa" in Idoma, "oda" in Igala, "chiha" in Tiv and "abwa" in Igbo (Dalziel, 1937). This plant is traditionally used in the management of breast tumours, vestibule vaginal fistula, swellings and abscesses (Survey Report, 1998). A concoction from the stem bark is used in diabetes and sickle cell disorders (NCAC Policy and Operational Guidelines, 1992). Its stem bark and leaves produce a liquid called oleoresin which has been used in traditional medicine for more than 400 years (Gilbert, 2000) and it contains a large but varying amounts of volatile oils which are used in traditional medicine as an anti-inflammatory agent and in the treatment of variety of genito-urinary tract diseases and skin ailments (Raffauf, 1992). Furthermore, it is also used as an anti-rheumatic, antibacterial, diuretic, hypotensive agent, laxative, purgative, expectorant, vermifuge and vulnerary (Fleury, 1997).

It is not known whether the stem bark of *D. oliveri* contains any bioactive agent which can modulate mitochondrial-mediated cell death and thus become useful in drug development in diseases where apoptosis is dysregulated. It is in this regard that we investigated the effects of some solvent fractions of the crude extract of the stem bark of the plant on mPT formation and mitochondrial-dependent cell death.

MATERIALS AND METHODS

Chemicals and Reagents: All reagents were of the highest purity grade available and were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemicals and assay kits were of pure form and analytical grade.

Experimental Animals: Male Wistar strain rats weighing between 100 – 120 g were obtained from the Pre-Clinical Animal House, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Nigeria. The animals were allowed to acclimatize for 14 days in the Animal House, Department of Biochemistry, University of Ibadan before the commencement of the experiments. All animals had access to water and chow *ad libitum* and were kept under standard conditions of temperature and humidity. Rules guiding animal studies as stipulated by the Ethical Committee of University of Ibadan were followed. These rules are similar to international guidelines on animal handling.

Preparation of plant extract and fractions: Freshly harvested stem of *D. oliveri* was obtained from the Botany Department of University of Ibadan, Oyo State, Nigeria. Botanical identification and authentication were carried out at the Department of Botany, Faculty of Science, University of Ibadan, Ibadan, Nigeria and a voucher number (UIH: 22383) was deposited in the herbarium. The stem barks were washed and peeled and air-dried for 3 weeks in the laboratory after which they were ground into powdered and weighed. The air-dried powder (1 kg) was soaked with sufficient ethanol in an all-glass jar at room temperature for 72 h and the filtrate concentrated at 40^0C under reduced pressure (Stuart Rotavapor) to obtain the crude ethanol extract of *Daniellia oliveri* (EEDO) stem bark.

Partitioning of crude EEDO using vacuum liquid chromatography: The crude EEDO (3 g) was pre-absorbed with silica gel 60 (0.040– 0.063 mm, MERCK). The mixture was air-dried to obtain a powder. A sintered funnel for vacuum liquid chromatography was packed with 6 g of this silica gel. Solvents were passed through the funnel in order of increasing polarity; n-hexane, chloroform, ethylacetate and ethanol fractions were obtained in this order. All eluted fractions were concentrated to dryness under reduced pressure at 40^0C to obtain the various solvent fractions, namely n-hexane (HFDO), chloroform (CFDO), ethylacetate (EAFDO) and ethanol (EFDO), of the crude extract, and were stored in glass vials at 4^0C until use.

Preparation of low-ionic-strength mitochondria: Low-ionic-strength liver mitochondria were isolated from male albino rats using the procedure described by Johnson and Lardy (1967) and as modified by Olorunsogo *et al.*, (1979). The animals were sacrificed by cervical dislocation and the livers were excised and trimmed to remove excess tissue. The livers were weighed, washed with homogenizing buffer (210 mM mannitol, 70 mM sucrose, and 5 mM HEPES-KOH at pH 7.4) and 1 mM ethylene glycol tetraacetic acid, and homogenized as a 10% suspension in ice-cold buffer. The homogenate was centrifuged twice in a MSE angle 13 refrigerated centrifuge at 2300 rpm for 10 minutes twice to remove the cellular debris. The resulting supernatant was centrifuged at 13,000rpm for 10 minutes to pellet the mitochondria, which were washed with washing buffer (210 mM mannitol, 70 mM sucrose, 5 mM HEPES at pH 7.4 and 0.5% Bovine serum albumin) at 12,000 rpm for 10 minutes. The mitochondria obtained were immediately resuspended in a small quantity of mannitol, sucrose and HEPES buffer (210 mM mannitol, 70 mM sucrose, and 5 mM HEPES–KOH at pH 7.4) and dispensed into Eppendorf tubes and kept on ice. The mitochondria were used within 4 h of isolation.

Mitochondrial swelling assay: In this study, permeability transition was assessed by measuring the swelling of mitochondria by monitoring the associated decrease in light scattering. The mPT was monitored by the method of Lapidus and Sokolove (1993) by measuring changes in absorbance of mitochondrial suspension in the absence and presence of exogenous calcium. Intact mitochondria (0.4 mg/mL) were pre-incubated in the presence of 0.8 μM rotenone and MSH buffer for 3.5 minutes prior to the addition of 5 mM sodium succinate. In order to assess Ca^{2+}-induced swelling, mitochondria were pre-incubated in 0.8 μM rotenone and MSH buffer for 3minutes. Ca^{2+} was then added to the reaction mixture while sodium succinate was added 30 seconds later in a total reaction volume of 2.5 mL. Spermine was used as the standard inhibitor of mitochondrial swelling, and was added prior to mitochondrial pre-incubation with rotenone. Change in absorbance was estimated at 540nm at 30 seconds interval for 12 minutes in a T70 UV-visible spectrophotometer, PG Instrument Ltd. To determine the effect of the extracts of

D.oliveri on Ca^{2+}-induced mPT pore opening, different concentrations of each of the extract fractions were separately pre-incubated with mitochondria for 3 minutes following the addition of Ca^{2+} to the reaction mixture. But calcium was excluded in the reaction mixture when the direct modulatory effect of the extracts on intact mitochondria was desired. Mitochondrial swelling was measured as decrease in absorbance at 540nm. Swelling rate was quantified as 540/min/mg. Mitochondrial protein was estimated according to the method described by Lowry *et al* (1951) using bovine serum albumin (BSA) as standard.

Determination of mitochondrial ATPase (mATPase) activity and lipid peroxidation: Liver mATPase activity was determined by the method described by Olorunsogo and Malomo (1985). Estimation of inorganic phosphate released was measured according to the procedure described by Bassir (1963) and as modified by Olorunsogo and Bababunmi (1979). A modified Thiobarbituric Acid Reactive Species (TBARS) assay method was deployed to measure the lipid peroxide formed from mitochondrial membrane lipid peroxidation using the method of Ruberto *et al* (2000). Percentage inhibition of lipid peroxidation by the extract was calculated from the absorbance value of the fully oxidized control and that in the presence of extract using the formula (E-C/C) * 100

Assay for cytochrome c release: The quantification of cytochrome c release from isolated mitochondria was performed by measuring the Soret (c) peak from cytochrome c at 414 nm (e = 100 mmol/L/cm]), according to method of Appaix *et al.* (2000). Mitochondria (1 mg protein/mL) were pre-incubated in the presence of 0.8 mmol/L rotenone in a medium containing 210 mM mannitol, 70 mM sucrose and 5 mM HEPES–KOH (pH 7.4) for 30 minutes at 27^{0}C in the presence of different concentrations of the fraction and using 24 mM calcium as the standard triggering agent. After the incubation, the mixture was centrifuged at 15,000 rpm for 10 minutes. The optical density of the supernatant was measured at 414 nm which is the Soret (c) peak for cytochrome c.

Statistical analysis: Data are presented as mean ± standard deviation of triplicate determinations. The values were analyzed using one-way/two way analysis of variance with the the aid of GraphPad Prism (version 5.0)

RESULTS

Results of the phytochemical screening of different solvent fractions of *Daniellia oliveri* stem bark are depicted in Table 1. The results show that cardiac glycosides, flavonoids, saponins, tannins, glycosides, coumarins, phlobatannins, terpenoids and steroids are present in the stem bark. Specifically, the crude extract (EEDO) and ethanol fraction (EFDO) contain several of the phytochemicals screened, while chloroform (CFDO) and ethyl acetate (EAFDO) fractions contain only few of these phytochemicals.

Figure 1 illustrates the results of effects of Ca^{2+} and spermine on intact rat liver mitochondria. In the absence of calcium, there was no appreciable change in absorbance of mitochondria respiring on succinate in the presence of rotenone, showing that the isolated mitochondria were intact. However, addition of exogenous Ca^{2+} to intact mitochondria significantly induced the opening of the mPT pore by 18 folds relative to control. Addition of spermine, a standard mPT pore inhibitor reversed the opening approximately by 75%. Taken together, exogenous Ca^{2+} significantly induced mPT pore opening while spermine inhibited the observed calcium-induced opening. This indicated that the mitochondrial were intact and not uncoupled and therefore suitable for further use.

Table 1:
Phytochemicals present in the Crude ethanol extract/ solvent fractions of *D. oliveri* stem bark

Phytochemicals	Crude extract (EEDO)	Ethanol (EFDO)	Ethylacetate (EAFDO)	Chloroform (CFDO)
Saponins	+	+	-	-
Flavonoids	+	+	-	-
Alkaloids	+	+	-	-
Terpenoids	+	+	+	+
Cardiac glycosides	+	+	+	+
Anthraquinones	+	+	-	-
Tannins	+	+	+	-
Steroids	+	+	+	+
Reducing sugar	-	-	-	-
Coumarin	+	-	+	-
Phlobatannins	+	+	+	-

+ = present, - = absent

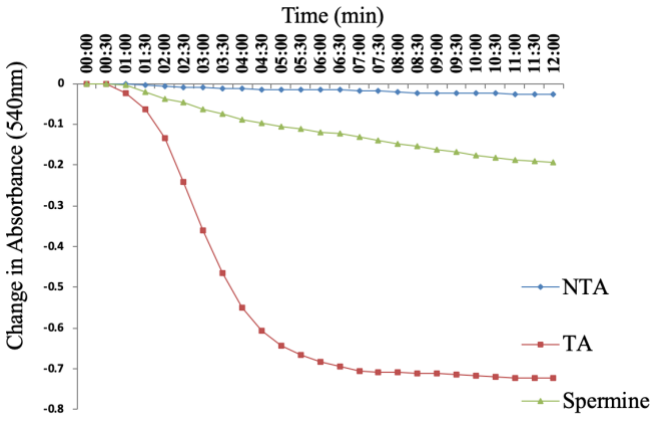

Figure.1
Calcium-induced mitochondrial membrane permeability transition pore opening in mormal rat liver mitochondria and its reversal by spermine
NTA: Non Triggering Agent, TA: Triggering Agent

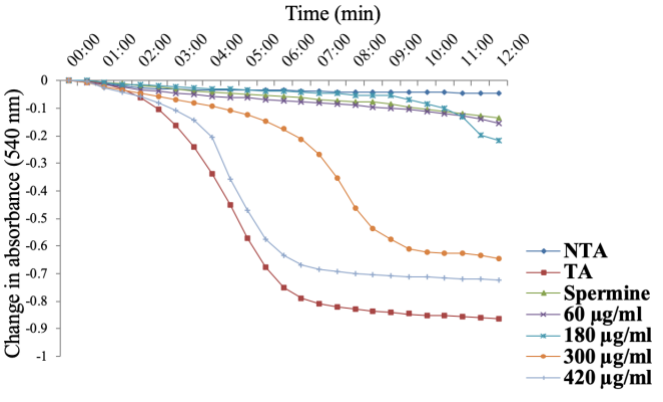

Figure 2:
Effects of varying concentrations of the crude Ethanol of *D. oliveri* (EEDO) on rat liver mPT pore (*in vitro)* in the absence of TA. NTA: Non Triggering Agent, TA: Triggering Agent

Figures 2 and 3 show the representative profiles of changes in absorbance of mitochondria respiring on succinate in the presence of rotenone on the addition of varying concentrations of the crude Ethanol Extract of *Daniellia oliveri* (EEDO) for a period of 12 minutes both in the absence and presence of calcium, the triggering agent, respectively. Exposure of mitochondria to varying concentrations (60, 180, 300, 420 µg/ml) of EEDO in the absence of calcium induced opening of the mPT pore by 3, 4.2, 13.7 and 15.4 folds, respectively. In this regard, the inductive effect was concentration-dependent. Exposure of the intact mitochondria to similar concentrations of EEDO in the presence of calcium slightly inhibited calcium-induced opening of the mPT pore by 10.3, 11.3, 14.4 and 18.9%, respectively while spermine inhibited the pore by approximately 70%.

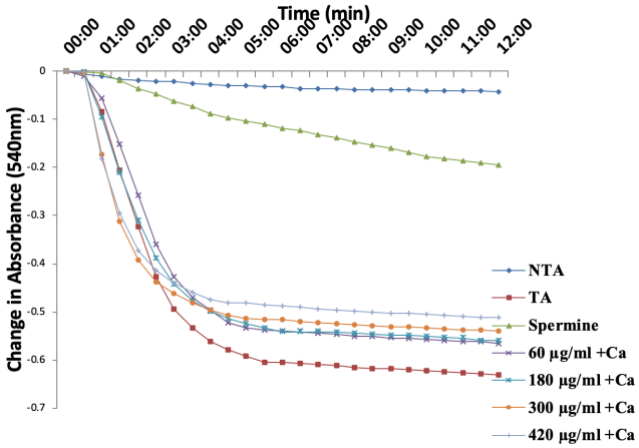

Figure 3:
Effects of crude ethanol of *D. oliveri* on intact rat liver mPT pore *in vitro* in the presence of TA. NTA: Non Triggering Agent, TA: Triggering Agent

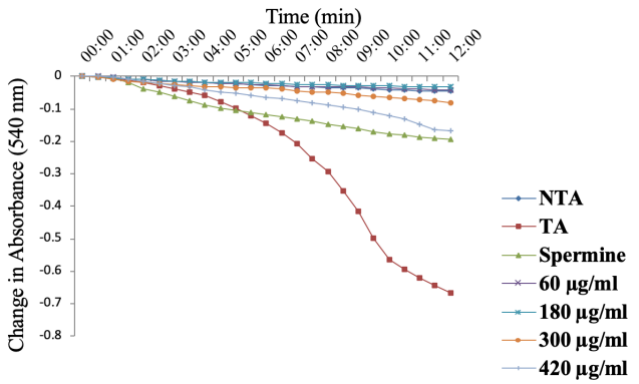

Figure 4:
Effects of varying concentrations of Chloroform fraction of *D. oliveri* (CFDO) stem bark on rat liver mPT pore (*in vitro)* in the absence of TA
TA: Triggering Agent, NTA: Non Triggering Agent

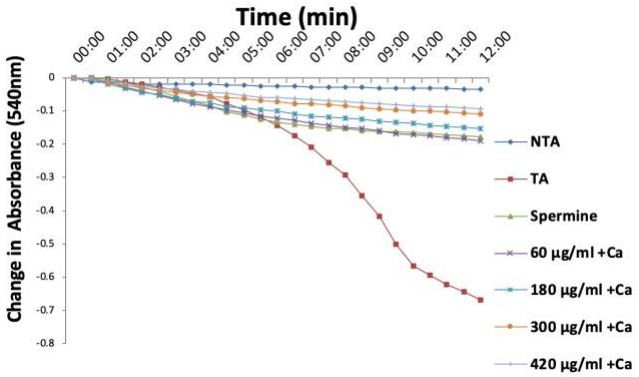

Figure 5:
Effects of varying concentrations of Chloroform fraction of *D. oliveri* (CFDO) stem bark on rat liver mPT pore (*in vitro)* in the presence of TA
NTA: Non-Triggering Agent, TA: Triggering Agent

Figures 4 and 5 summarize the effects of CFDO stem bark on intact mPT in the absence and presence of calcium, respectively. The data obtained reveal that in the absence of calcium, all concentrations tested had no significant effect on the mPT pore except at the highest concentration (420 µg/mL) which gave and inductive fold of 2.7. However, CFDO (60-420 µg/mL) strongly reversed calcium-induced mPT pore opening in a concentration –dependent manner by 71, 77, 83, and 86%, respectively while the inhibition by spermine was 73%.

Figure 6 and 7 depict the effects of varying concentrations of EAFDO on mPT pore in the presence and absence of calcium. Similar to the data obtained for exposure to CFDO, only the highest concentration (420µg/ml) of EAFDO exhibited an inductive effect of 3.4 folds while lower concentrations had no effect whatsoever on mPT pore in the absence of calcium. Calcium-induced pore opening was slightly inhibited by concentrations of EAFDO with the highest concentration producing 37% inhibition of the mPT pore as opposed to 81% (spermine), the standard inhibitor.

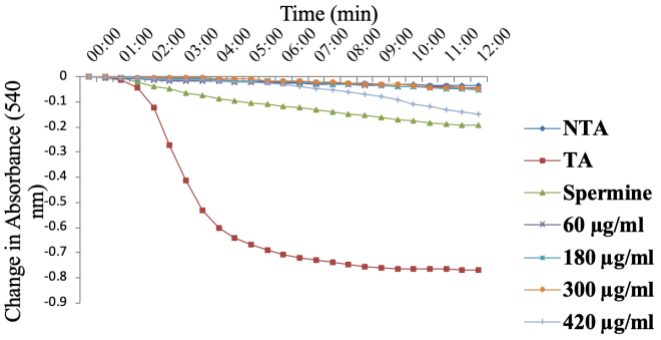

Figure 6:
Effects of varying concentrations of Ethylacetate fraction of *D. oliveri* (EFDO) stem bark on rat liver mPT pore *in vitro* in the absence of TA. NTA: Non Triggering Agent, TA: Triggering Agent

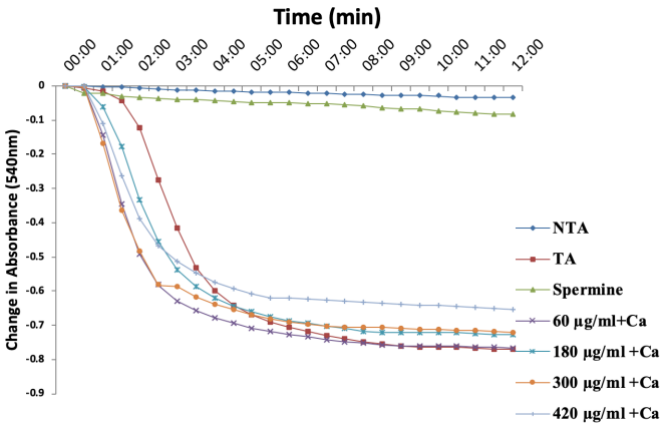

Figure 7:
Effects of Ethyl acetate fraction of *D. oliveri* stem bark on rat liver mPT pore *in vitro* in the presence of TA. TA: Triggering Agent, NTA: Non Triggering Agent

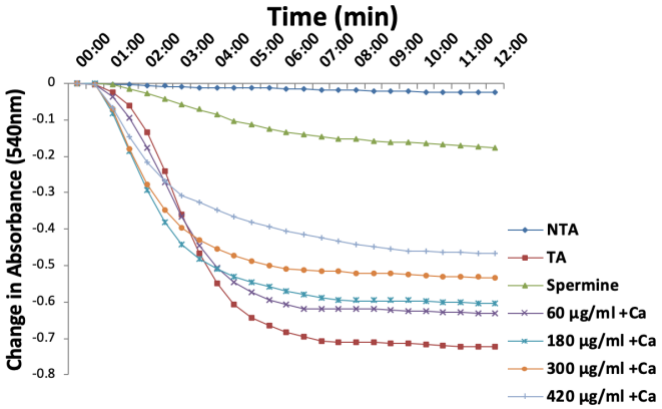

Figure 9:
Effects of Ethanol fraction of *D. oliveri* stem bark on rat liver mPT pore *in vitro* in the presence of TA .
NTA: Non Triggering Agent, TA: Triggering Agent

Figures 8 and 9 present the profiles obtained when varying concentrations of EFDO was added to mitochondrial suspension in the absence and presence of calcium. In the absence of calcium, lower concentrations of EFDO did not have any significant effect but significantly induced pore opening at higher concentrations of 300 and 420 µg/mL by 3.6 and 12.2 folds, respectively. These results show that EFDO is the most potent fraction which induced opening of the mPT pore. In the presence of calcium, varying concentrations of EFDO slightly inhibited calcium-induced mPT pore opening by 12, 16, 26 and 36%, respectively.

Figure 10 shows the effects of varying concentrations of solvent fractions of DO stem bark on rat liver mitochondrial ATPase (mATPase) activity at physiological pH (7.4). The results showed that of all the solvent fractions tested, EFDO significantly enhanced mATPase activity in a concentration - dependent manner with enhancement effect of 63% at the highest concentration (420 µg/mL) which was close to that of 2,4-dinitrophenol,the standard uncoupler of oxidative phosphorylation (68%). Average enhancement effects of 48 and 46% were obtained with CFDO and EAFDO at the highest concentration of 420 µg/mL. This further confirms our previous data that showed that EFDO is the most potent of all fractions with respect to induction of mPT pore opening.

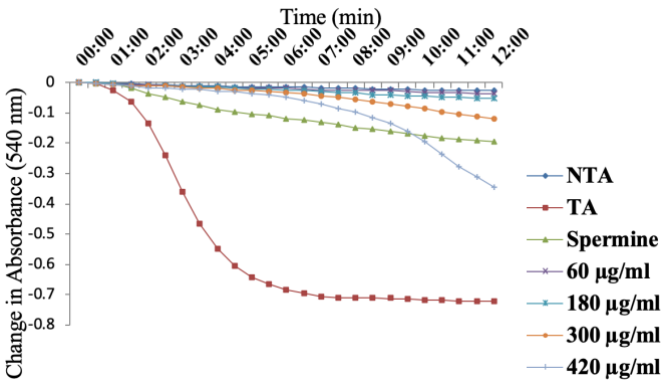

Figure 8:
Effects of varying concentrations of Ethanol fraction of *D. oliveri* (EFDO)stem bark on intact rat liver mPT pore *in vitro* in the absence of TA. NTA: Non Triggering Agent. TA: Triggering Agent

The effect of solvent fractions of *D. oliveri* stem bark on Fe^{2+} induced lipid peroxidation of rat liver mitochondria is presented in figure 11. The various solvent fractions inhibited generation of lipid peroxides to varying degrees in a concentration-dependent pattern with EFDO eliciting the highest inhibitory effect. In this regard, CFDO, EAFDO and EFDO inhibited lipid peroxides generated by $FeSO_4$ at the highest concentration (12mg/mL) by 58, 114 and 133%, respectively.

Figure 12 shows a remarkable and concentration-dependent release of cytochrome C when varying concentrations of EFDO were added to mitochondria with respect ot other fractions. Approximately 0.7, 0.9 and 1.5 nmol/mg protein of cytochrome c were released into the cytosol when CFDO, EAFDO and EFDO were added to intact rat liver mitochondria while addition of exogenous calcium as standard pore inducer elicited the release of about 1.6 nmol/mg protein of cytochrome c. These data further corroborate the observation that the EFDO is the most potent in induction of mPT pore leading to the release of the apoptogenic protein, cytochrome c

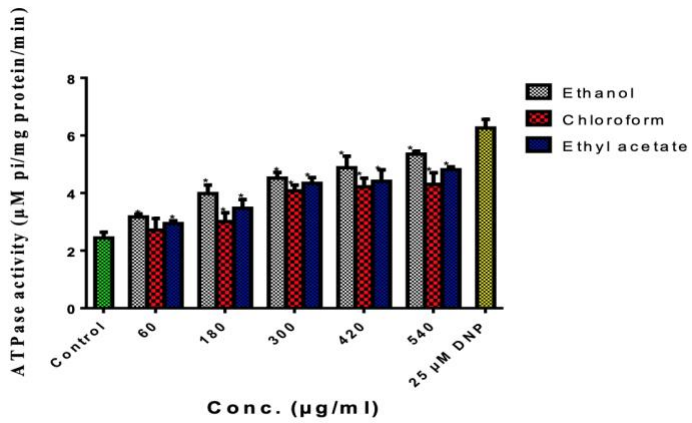

Figure 10:
Effects of varying concentrations of different activity solvent fractions of *D.oliveri* stem bark on rat liver mitochondrial ATPase at physiological pH (7.4)

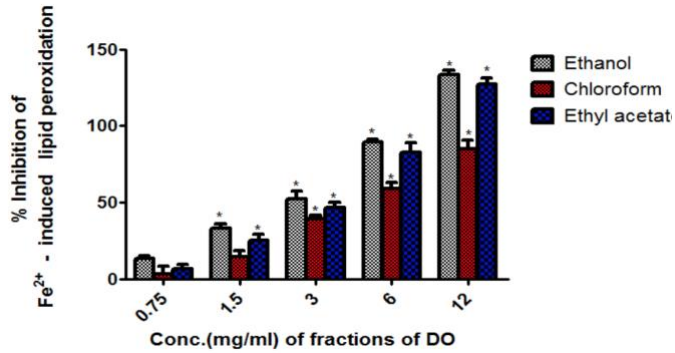

Figure 11:
Percentage inhibition of Fe^{2+}- induced lipid peroxidation of normal mitochondria in the presence of varying concentrations of solvent fractions of *D. oliveri* stem bark

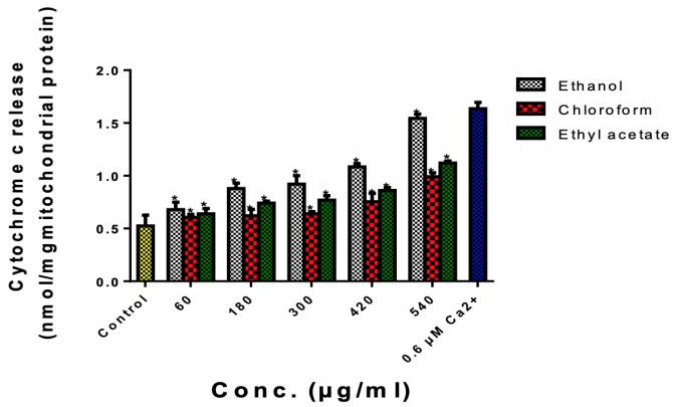

Figure 12:
Pattern of the extent of cytochrome C release in normal rat liver mitochondria in the presence of different concentrations of solvent fractions of DO

DISCUSSION

Cell death is an important component of normal physiology, maintaining homeostasis by balancing cell production with cell demise (Hinsull and Bellamy, 1981; Cooper and Youle, 2012). Dysregulation of cell death has been associated with the pathophysiology of several diseases including stroke, cancer, diabetes, cardiovascular and neurodegenerative disorders (Lockshin and Zakeri, 2007). Medicinal plants have a long standing role in the management of these diseases and there is a sudden resurgence in the acceptance of the use of herbal medicine because of the toxicities associated with synthetic drugs owing to offsite targets (Mahomoodally, 2013; Jamshidi-Kia *et al.*, 2018)

The opening of the inner membrane mitochondrial permeability transition (mPT) pore is one of the causes of mitochondrial Permeability Transition, a process that compromises the bioenergetic status of the cell and initiates its death (Crompton, 1999; Bernadi and Di Lisa, 2015). The activation of the mPT pore as well as its inhibition has been implicated in the etiology of many diseases including cancer, neurodegerative diseases as well as other age–associated diseases (Bonora and Pinton, 2014; Šileikytė and Forte, 2019). Inhibition of the opening of the pore represents a cytoprotective approach in Ischaemic-reperfusion injury and neurodegenerative diseases characterized by excessive cell death while the activation/induction of this opening is beneficial in the treatment of most tumors arising from inadequate cell death (Najafi *et al.*, 2014; Bonora and Pinton, 2014).

Interestingly, mPT pore has been shown to play a role in programmed cell death (apoptosis) as well as the necrotic form of cell death which is accidental (Zamzami *et al.*, 2005; Rasola and Bernardi, 2011). Its role in cell death is underscored by the highly orchestrated series of biochemical events including enhancement of mitochondrial ATPase activity (reduction in ATP levels) and release of cytochrome c, which results from the opening of the mPT pore (Bernadi and Di Lisa, 2015). Given that the mPT pore plays an important role in diseases associated with dysregulated cell-death, it represents a major therapeutic target that can be influenced by several compounds (Su *et al.*, 2013). Hence, the search for bioactive agents that can modulate the opening of the mPT pore is on the rise.

In this study, the modulatory effects of different solvent fractions of the stem bark of *Daniellia oliveri* on mitochondrial-dependent cell death was evaluated by measuring a number of cell death parameters associated with the mitochondria including mitochondrial swelling (opening of the mPT pore). Opening of the mPT pore was measured by determining the susceptibility of the pore to exogenous calcium which results in mitochondrial swelling which is measured by a decrease in light scattering effect. The decrease in absorbance is usually reversed by spermine or cyclosporine A, a standard inhibitor of mPT.

Our results show that the mPT pore opened in normal mitochondria in the presence of exogenous calcium and that spermine reversed this effect of calcium thus indicating that the mitochondria were intact and suitable for further use. Interestingly, in the absence of calcium, there was significant opening of the pore when different concentrations of crude EEDO was exposed to intact mitochondria. Furthermore, of all the fractions of crude EEDO tested, ethanol fraction (EFDO) had significant inductive effects at higher concentrations of the fractions. This reveals that bioactive agents that could interact with the pore thereby inducing opening of the pore are abundant in the EEDO and EFDO. These fractions may therefore have potential in management of diseases caused by inadequate apoptosis. The observed inductive effect was reversible by spermine which is an indication that the bioactive agent present in *D.oliveri* did not compromise the integrity of the membrane while activation of pore opening occurred. The strong inhibition of generation of iron-induced lipid peroxides by fractions of *D. oliveri* attests to the protective effect of these fractions on membrane integrity. The other fractions (CFDO and EAFDO) had no effect whatsoever on the mPT pore at lower concentrations although a mild inductive effect was recorded at the highest concentration of the fractions. This shows that bioactive agents that induce opening of the pore are very low in these fractions.

In the presence of calcium, all fractions except CFDO reversed calcium-induced opening of the mPT pore minimally. Interestingly, CFDO significantly inhibited calcium-induced opening of the pore with the highest concentration producing a greater inhibitory effect (86%) than spermine (73%), a standard inhibitor of pore opening. The observation that CFDO possesses a strong inhibitory effect on the opening of the mPT pore is an indication that it contains bioactive compounds that are capable of preventing large amplitude swelling caused by addition of exogenous calcium and explains why it had no significant inductive effect on the mPT pore in the absence of calcium. This fraction could

therefore find use in diseases characterized by excessive cell death/tissue wastage. These results lay credence to the fact that the mPT pore is a selective target which can be manipulated for the treatment of diseases (Su *et al.,* 2013). Furthermore, they confirm the fact that different bioactive agents are distributed in different solvent fractions of medicinal plants and corroborates the traditional use of the *D. oliveri* in the management of breast tumors (inadequate cell death) (Survey Report, 1998) diabetes and sickle cell disorders (excessive cell death) (NCAC Policy and Operational Guidelines, 1992). Hence, the polar fraction (EFDO) elicits inductive potential on the pore while the non-polar fraction (CFDO) of the same plant has a strong inhibitory effect on the mPT pore.

To further confirm our results, we assessed mitochondrial ATPase activity and release of cytochrome c upon exposure to the different solvent fractions of the plant. This is because opening of the mPT pore causes the mitochondrial ATP synthase to work in the reverse direction, hydrolyzing ATP instead of synthesizing it (Chinopoulos and Adam-Vizi, 2012) owing to the release of cytochrome c into the cytosol. In addition, recent evidence suggest that mATPase remains a strong candidate as the mPT pore (Bonora *et al.*, 2013, Bernadi, 2018).

In the mitochondria, cytochrome c functions as an electron carrier in oxidative phosphorylation while its release into the cytosol represents a point of no return for cell death to take place. Hence, the release of inorganic phosphate is an index of activation of mitochondrial ATPase activity/ATP consumption and confirms the opening of the mPT pore. Our data reveals that EFDO significantly enhanced mATPase activity at physiologic pH when compared with control and other fractions with evidence of release of significant amounts of cytochrome c in the cytosol. Besides, there is accumulating evidence that high inorganic phosphate levels induce mitochondrial-dependent cell death (Seidlmayer *et al.,* 2010). Our data indicate that the release of inorganic phosphate as a result of the interaction of the bioactive agents in *D.oliveri* with mPT pore components contributes to the persistent opening of the pore to bring about the release of the apoptogenic protein, cytochrome c.

Put together, *D. oliveri* contains a diverse array of bioactive agents as shown by its phytochemical constituents. These agents have potential of modulating the opening of the mPT pore and could be useful in exploiting mitochondrial vulnerability to modulate cell death in the management of diseases associated with defective cell death pathways, thereby maintaining normal tissue homeostasis.

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