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Research Article

Fractions of *Ageratum conyzoides* Induce Cytotoxicity in Rat Liver Cells Via Mitochondrial Permeability Transition Pore Opening: Potential Anti-cancer Agents

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ABSTRACT

Mitochondrial Permeability Transition (mPT) pore has become a target for the development of cytotoxic drugs that are relevant in situations of deregulated apoptosis. This study therefore investigated the effects of various fractions of the methanol extract of Ageratum conyzoides, a medicinal plant, on mPT pore. The methanol extract of Ageratum conyzoides (MEAC) was partitioned in succession between n-hexane, chloroform, ethylacetate and methanol. The fractions were concentrated at 40ºC to obtain chloroform (CFAC), ethylacetate (EFAC) and methanol (MFAC) fractions. Isolated rat liver mitochondria were exposed to the fractions. The opening of the pore, cytochrome c release, mitochondrial ATPase activity and lipid peroxidation were assessed spectrophotometrically. The study showed that all the fractions induced the opening of the pore with CFAC being the most potent. All the fractions caused cytochrome c release, enhanced mitochondrial ATPase activity and inhibited lipid peroxidation with CFAC having the highest effect. These findings therefore suggest that fractions of Ageratum conyzoides induce cytotoxicity in rat liver cells via mPT pore opening and also possess antioxidant property with CFAC being the most potent. The fraction will therefore be subjected to further study as this may be relevant in cases where apoptosis needs to be up regulated.

Keywords: *Mitochondrial permeability transition pore, apoptosis, Ageratum conyzoides, cancer*

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INTRODUCTION

Apoptosis is a programmed physiological process by which undesirable cells are systematically removed by reasons of signals received (Du et al., 2001; Green and Llambi 2015; Yan et al. 2020). Dysregulated apoptotic signaling has been a major factor in many human diseased conditions especially neurodegenerative diseases and cancers (Elmore, 2007; Gibellini and Moro, 2021). Mitochondria have been shown to play significant roles in apoptosis by regulating the release of cytochrome c and other pro-apoptotic factors (Nicholson and Thornberry, 2003; Gulbins et al. 2011).

Apoptosis occurs mainly by two well-known pathways in cells; the death receptor-mediated and mitochondrial-mediated pathway (Lockshin and Zakeri, 2004; Danial and Korsmeyer, 2004; Goldar et al., 2015). The mitochondrial-mediated pathway depends primarily on the permeabilization of mitochondrial membrane which leads to loss of mitochondrial trans-membrane potential, release of apoptogenic mitochondrial proteins, activation of caspase 9, subsequent cleavage of caspases 3, 6, or 7 and ultimately, execution of apoptosis (Reed, 2004; Guimarães and Linden, 2004; Green and Llambi, 2015). Cytotoxicity could be assayed based on several cell functions especially mitochondrial membrane permeability, enzyme activity and ATP production (Özlem, 2017).

Experimental evidences have shown that some bioactive agents in medicinal plants elicit their chemoprotective effect against cancers and tumors by the induction of mitochondrial-mediated apoptosis via mitochondrial permeability transition (mPT) pore opening (Olowofolahan et al., 2015; Niloufar et al., 2017; Olowofolahan et al., 2019). Curcumin (Chan et al., 2005), Resveratrol (Ungvari et al., 2009) and Quercetin (Gibellini et al., 2011) are potent natural compounds that have been shown to modulate mPT.

Ageratum conyzoides is an herbaceous annual plant found in North and Central America as well as other countries in tropical regions. Its folkloric use includes treatment of cancer (Wijayakusuma and Dalimarta,1994), uterine problems (Rajwar, 1983), wounds and leucorrhoea (Katewa,1997), ulcers, diarrhea, skin diseases, etc. (Ming, 1999; Rosangkima and Prasad, 2001). Studies have shown that Ageratum conyzoides possesses antioxidant and anticancer property

(Adebayo et al., 2010; Acheampong et al., 2015). Its antidiabetic, anti-inflammatory and wound healing property has also been documented (Masengo et al., 2015). However this study was aimed at investigating the effect of various fractions of the plant on induction of mPT pore, cytochrome c release, mitochondrial ATPase activity and lipid peroxidationin order to determine the most potent fraction.

MATERIALS AND METHODS

Chemicals and reagents: All reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Plant material: Fresh leaves of *Ageratum conyzoides* were obtained from a local farmland in Ibadan, Oyo State, Nigeria. Botanical identification and authentication of the plant was done by Mr Esimekhuai, D.P.O. at the herbarium unit, Department of Botany, University of Ibadan, Nigeria, with voucher number UIH-22958. The fresh plant was washed, shade-dried for about three weeks and then pulverized to a powder. Six kilogramme air-dried, powdered leaf of *Ageratum conyzoides.* were extracted with 20 Liters of distilled methanol (Sigma Aldrich Chemical) in all- glass jars at room temperature for seventy-two hours. The filtrate was decanted, filtered and concentrated under reduced pressure using a rotary evaporator (N-100, Eyla, Tokyo, Japan). The methanol extract concentrate was heated over a water bath at 40°C to obtain a solvent free extract. The percentage yield was 2.5%.

Preparation of the fractions: The methanol extract was further partitioned using vacuum liquid chromatography (VLC) technique. Silica gel 60 (0.040–0.063mm, MERCK) 10g was added to 15g of the methanol extract. The gel-sample mixture was stirred until a homogenous mixture was obtained. The mixture was air-dried to obtain a powder form. The sample was applied to the top of a prepared column after which it was eluted with n-hexane, chloroform, ethylacetate and methanol solvent in succession. The n-hexane was used to de-fat the methanol extract. The fractions obtained were concentrated at 40°C using rotary evaporator to obtain solvent free n-hexane (HFAC), chloroform (CFAC), ethyl acetate (EFAC) and methanol (MFAC) fractions. These fractions were concentrated to dryness and stored in the refrigerator until use. The methanol extract yielded 12.6%, 2.3%, 3.5% and 5.7%, for n-hexane, chloroform, ethylacetate and methanol fractions, respectively.

Experimental Animals: Male Wistar albino rats (80–90g) were purchased from the Preclinical Animal House, University of Ibadan, kept in clean cages and allowed to acclimatize for two weeks. The animals had free access to pelletized rat chow and water *ad libitum* throughout the experimental period. The work was conducted according to the guidelines of National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use.

Isolation of rat liver mitochondria: The isolation was carried out as described by Johnson and Lardy (1967), with little modification by Olorunsogo *et al*. (1979). The animals were sacrificed; liver was rapidly excised, trimmed, blotted with blotting paper and weighed. It was rinsed with isolation buffer (210 mM Mannitol, 70 mM Sucrose, 5 mM HEPES-KOH, pH 7.4 and 1 mM EGTA), and homogenized in a 10% w/v of ice-cold isolation buffer. The homogenate was centrifuged in an MSE refrigerated centrifuge (Progen Scientific, UK) at 2,300 rpm for five minutes to sediment nuclear fraction and cell debris. The supernatant obtained was centrifuged at 13,000 rpm for 10 minutes to obtain the mitochondrial pellet, which was washed twice, with washing buffer (210 mM Mannitol, 70 mM sucrose, 5 mM HEPES-KOH, pH 7.4, 0.5% BSA) at 12,000rpm for 10 minutes. The mitochondrial fraction was resuspended in suspension buffer (210 mM Mannitol, 70 mM sucrose, 5 mM HEPES-KOH, pH 7.4), dispensed into Eppendorf tubes as aliquots and stored at 4°C. All experiments with isolated mitochondria were performed within 4 hours of the preparation.

Determination of mitochondrial protein: This was done as described by Lowry et al. (1951), using bovine serum albumin as standard.

Determination of intactness of mitochondria: Mitochondrial Permeability Transition (mPT) was monitored by observing the changes in diffraction of light in the spectrophotometer through absorbance of mitochondria suspension at 540 nm in a T70 UV/Visible spectrophotometer (China) essentially according to the method of Lapidus and Sokolove (1993). Briefly, mitochondria (1 mg protein/ml) were pre-incubated in the presence of 0.8 µM rotenone (10 µl) in a medium containing 210 mM mannitol, 70mM sucrose and 5 mM HEPES-KOH (MSH) buffer (pH 7.4) (2,200 µl) for $3^1/2$ minutes at 27°C prior to the addition of 5 mM succinate (50 µl). The mPT was spectrophotometrically mornitored for 12 minutes at 30 seconds interval. The above experiment was repeated with mitochondria (1 mg protein/ml) pre-incubated in the presence of 0.8 µM rotenone (10 µl) in the same medium for 3 minutes at 27°C prior to the addition of 24 µM $CaCl_2$ (25 µl). Thirty seconds later, 5 mM succinate (50 µl) was added and mPT was quantified at 540 nm for 12 minutes at 30 seconds interval. This experiment was again repeated with mitochondria (1 mg protein/ml) pre-incubated in the presence of 0.8 µM rotenone (10 µl), 4 mM spermine (63 µl) in the same medium for 3 minutes at 27°C prior to the addition of 24 µM $CaCl_2$ (25 µl). Thirty seconds later, 5 mM succinate (50 µl) was added and mPT was quantified at 540 nm for 12 minutes at 30 seconds interval.

Mitochondrial swelling assay: Briefly, mitochondria (1 mg protein/ml) were pre-incubated in the presence of 0.8 µM rotenone (10 µl) in a medium containing 210 mM mannitol, 70mM sucrose and 5 mM HEPES-KOH (MSH) (pH 7.4) (2,200 µl) for 3 minutes at 27°C prior to the addition of different concentrations (8-72 µg/ml) of the various fractions of *Ageratum conyzoides*. Thirty seconds later, 5 mM succinate (50 µl) was added and mPT was quantified at 540 nm for 12 minutes at 30 seconds interval.

Assessment of mitochondrial F_oF_1 ATPase activity: This was determined by a modification of the method of Olorunsogo and Malomo (1985). Each reaction mixture

contained 65 mM Tris-HCl buffer (1300 µl) pH 7.4, 0.5 mM KCl (50 µl), 1 mM ATP (40 µl), 25 mM sucrose (50µl) and varying concentrations (8-72 µg/ml) of the fractions. The reaction mixture was made up to a total volume of 2 ml with distilled water. Mitochondrial suspension was added to the reaction medium in a shaker water bath and allowed to proceed for 30 minutes at 27°C. Aliquot amount (1mL) of 10 percent sodium dodecyl sulphate (SDS) solution was added to stop the reaction at 30 seconds intervals. 2, 4 Dinitrophenol (2, 4 DNP) (50 µl) was used as a standard uncoupling agent. Aliquot of each solution (300µl) was dispensed into fresh test tubes, followed by the addition of 300 µl of distilled water. To each of the test tube, 1 ml of 5% ammonium molybdate and 1 ml of 9% freshly prepared solution of ascorbic acid were added. The tube was well mixed and allowed to stand for 20 minutes. The absorbance was read at 680 nm. Water blank was used to set the spectrophotometer at zero.

Determination of cytochrome c release: The quantitative determination of cytochrome c released from isolated mitochondria was performed by measuring the Soret (γ) peak for cytochrome c at 414 nm (ε=100 mM^{-1} cm^{-1}), according to method of Appaix et al. (2000). Mitochondria (1 mg protein/ml) were preincubated in the presence of 0.8µM rotenone (10 µl) in a medium containing 210 mM mannitol, 70 mM sucrose and 5 mM HEPES-KOH (pH 7.4) (2,200 µl) for 30 minutes at 27 °C in the presence of different concentrations (8-72µg/ml) of the fractions, using 24 mM calcium (25 µl) 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 (γ) peak for cytochrome c.

Measurement of malondialdehyde (MDA) level: A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the MDA formed using mitochondria as lipid rich media (Ruberto et al., 2000). Mitochondria (1mg/ml protein) and varying concentrations of the fractions were added to each test tube and made up to 1 ml with distilled water; 0.05 ml of $FeSO_4$ (0.07 M) was added to induce lipid peroxidation and the mixture incubated for 30 minutes. Then, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% (w/v) thiobarbituric acid in 1.1% sodium dodecyl sulphate were added and the resulting mixture was vortexed and heated at 95°C for 60 minutes. After cooling, 3.0 ml of butan-1-ol were added to each tube and centrifuged at 3000 rpm for 10 minutes. The absorbance of the organic upper layer was measured at 532nm.

Statistical analysis of data: The data reported on mPT are representative of multiple (≥3) experiments using microsoft excel 2010. All other data were expressed as mean ± SD. Statistical analysis was performed using Graphpad Prism 6 software. Comparison of the variables was made using one-way analysis of variance (ANOVA). *P value < 0.05* was considered statistically significant.

RESULTS

Calcium-induced mitochondrial permeability transition pore opening in normal rat liver mitochondria and its reversal by spermine (inhibitor): Figure 1 displays the intactness of the mitochondrion that was used for the assay. As seen from the Figure, there was no significant change in absorbance of intact mitochondria over a period of twelve minutes. However, addition of calcium caused marked mPT pore opening which was significantly reversed by spermine, a standard inhibitor of mPT pore opening. The induction fold of calcium was 23.7 folds when compared to the NTA (control). The induction fold is calculated as:

{Change in absorbance of the test sample}÷{Change in absorbance of the NTA (control)}

Effect of various fractions of *Ageratum conyzoides* on mPT pore in the absence of calcium: Figure 2 represents the effect of MEAC on mPT pore. The results show that MEAC induced mPT pore opening having the highest induction fold of 5.5 at 72 µg/ml.

Representative profile of the evaluation of CFAC on the mPT pore in the absence of calcium is shown in Figure 3. The results showed that CFAC caused mPT pore opening with induction folds of 1.2, 1.9, 3.2, 9.1 and 16.9 at the same concentrations, when compared to NTA (control).

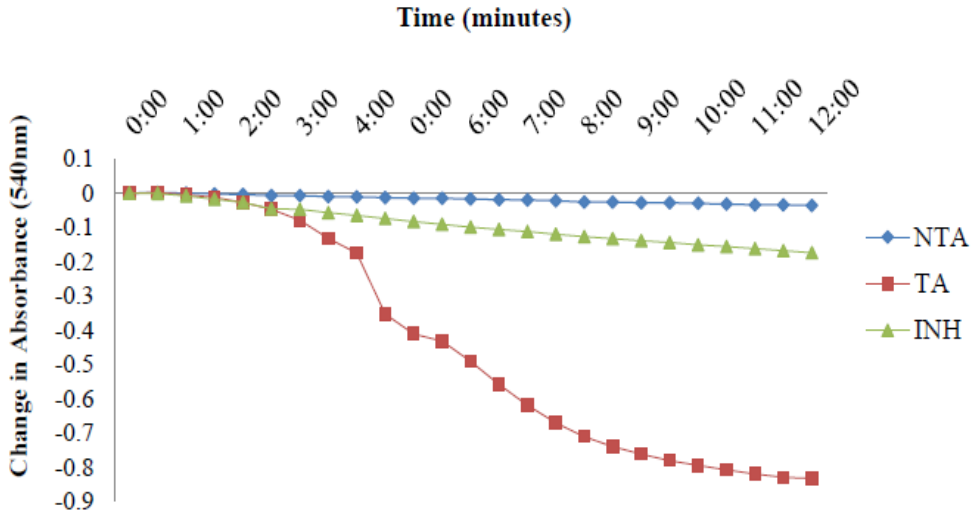

Figure 1: Calcium-induced mitochondrial permeability transition pore opening in normal rat liver mitochondria and its reversal by spermine
NTA: No triggering agent (control) TA: Triggering agent (calcium)
INH: Inhibitor (spermine)

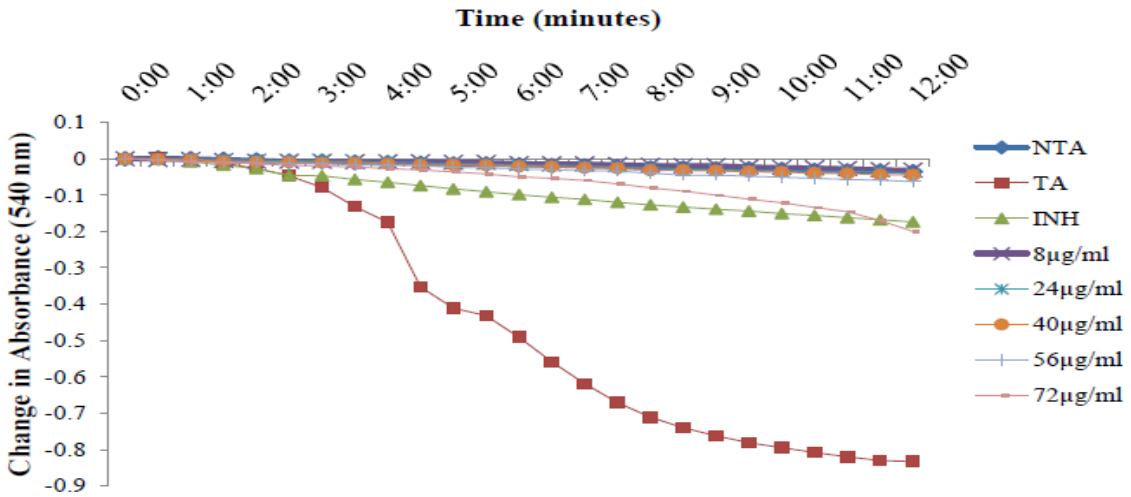

Figure 2:
Effects of varying concentrations of methanol extract of *Ageratum conyzoides* (MEAC) on rat liver mPT pore in the absence of calcium. ***Induction folds****: 0.9, 1.1, 1.3, 1.8, 5.5 compared to NTA (control)*

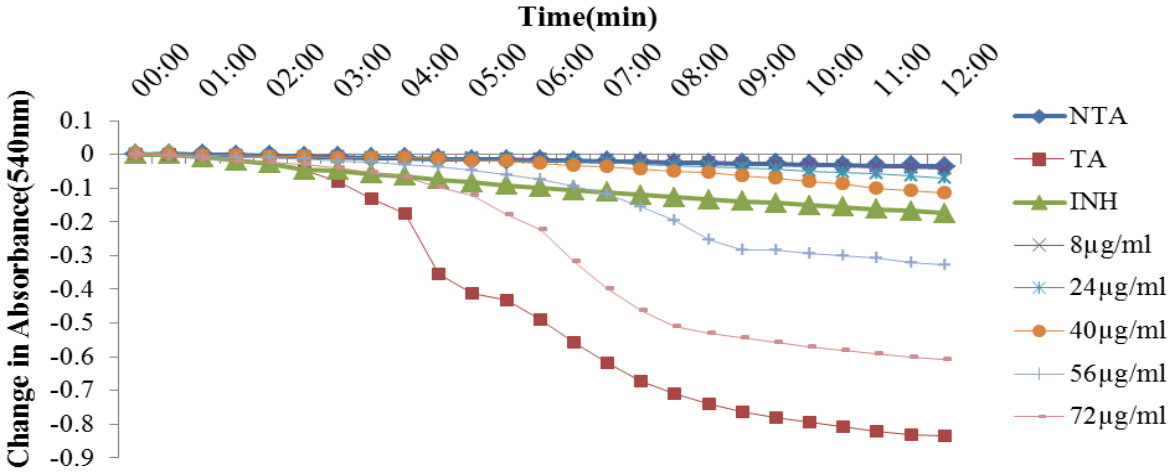

Figure 3:
Effect of varying concentrations of chloroform fraction of methanol extract of *A. conyzoides* (CFAC) on the mPT pore in the absence of calcium.
Induction folds: 1.2, 1.9, 3.2, 9.1 and 16.9 compared to NTA

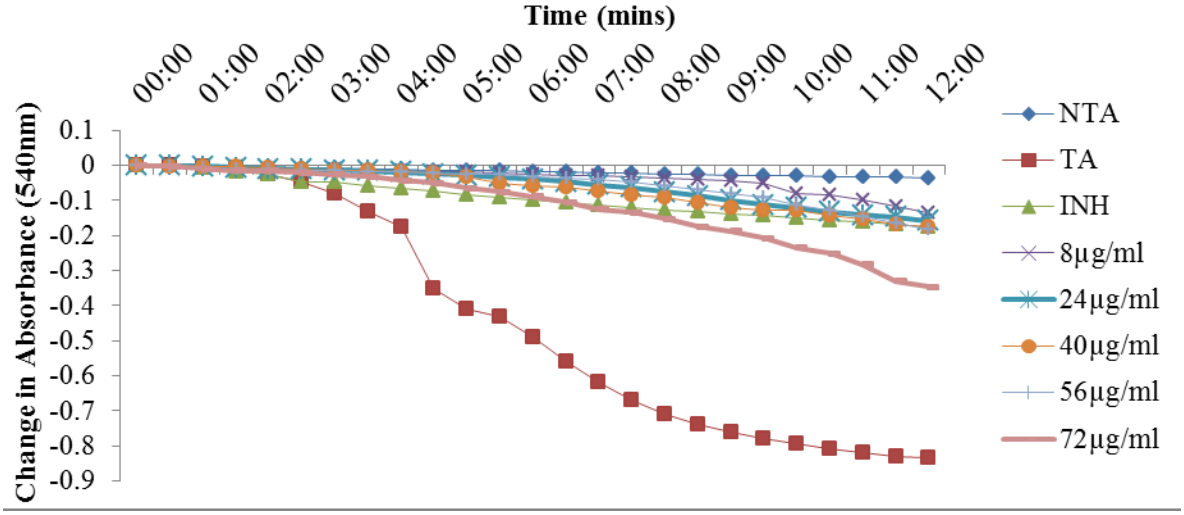

Figure 4:
Effect of varying concentrations of ethyl acetate fraction of *A. conyzoides* (EFAC) on the mitochondrial permeability transition pore in the absence of calcium.
Induction folds*: 3.7, 4.4, 4.9, 5.1, 10.4 compared to NTA*

Figure 4 shows the effect of EFAC on mPT pore in the absence of calcium. The EFAC also caused mPT pore opening with induction fold of 3.7, 4.4, 4.9, 5.1 and 10.4 at 8µg/ml, 24µg/ml, 40µg/ml, 56µg/ml and 72µg/ml, respectively, when compared to the NTA.

The MFAC showed mild inductive effect on mPT pore in the absence of calcium when compared with the NTA (Figure 5). However, all the fractions reversed calcium-induced mPT pore opening as depicted in Figures 6, 7, 8 and 9.

Effect of various fractions of *Ageratum conyzoides* on mPT pore in the presence of calcium: In this regard, MEAC at 8, 24, 40, 56 and 72µg/ml reversed calcium-induced opening respectively, by 20, 21, 24, 29 and 39% while CFAC at the same concentrations reversed pore opening by 19, 22, 24, 40 and 48%, respectively.

Similar concentrations of EFAC reversed calcium-induced opening by 50, 62, 76, 78 and 80% while MFAC at the same concentrations inhibited calcium-induced pore opening by 25, 28, 30, 32 and 50%. Spermine, a standard inhibitor of mPT pore opening showed 82% reversal.

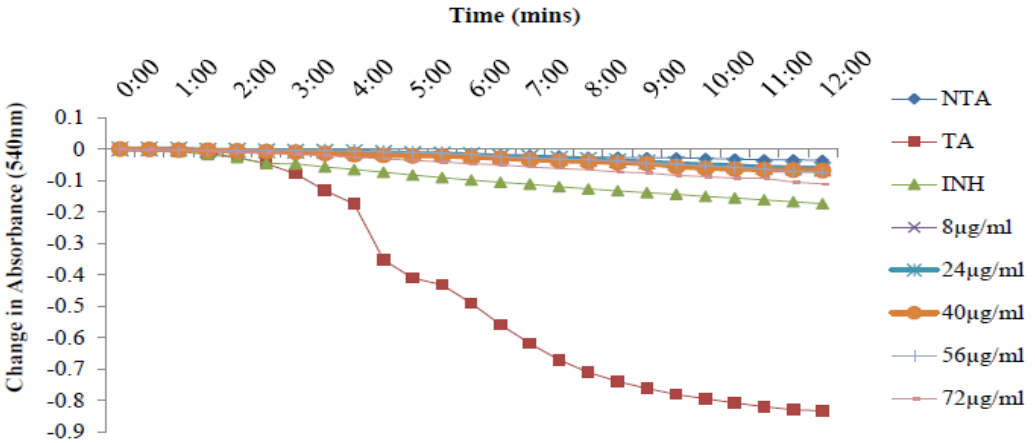

Figure 5:
Effects of varying concentrations of methanol fraction of methanol extract of *A. conyzoides* (MFAC) on rat liver mitochondrial permeability transition pore in the absence of calcium ***Induction folds**: 1.1, 1.8, 1.9, 2.1, 3.1 compared to the NTA.*

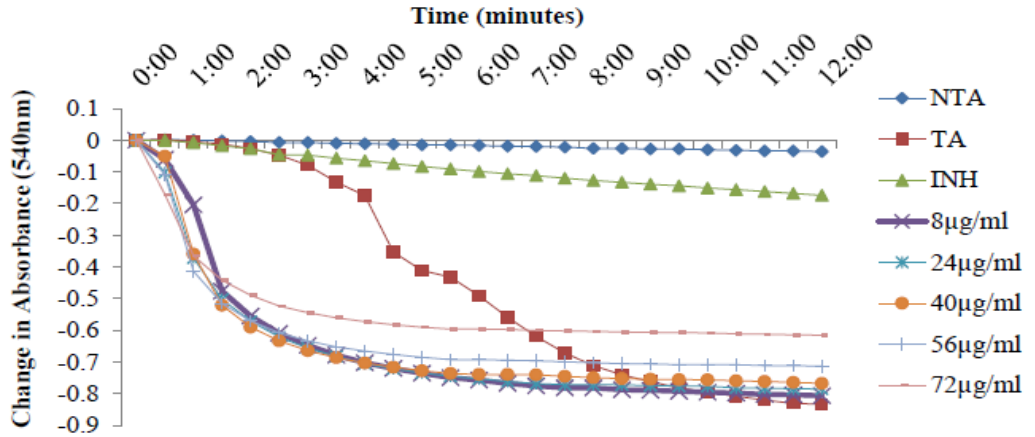

Figure 6:
Effects of varying concentrations of methanol extract of *Ageratum conyzoides* (MEAC) on rat liver mitochondrial permeability transition pore in the presence of calcium

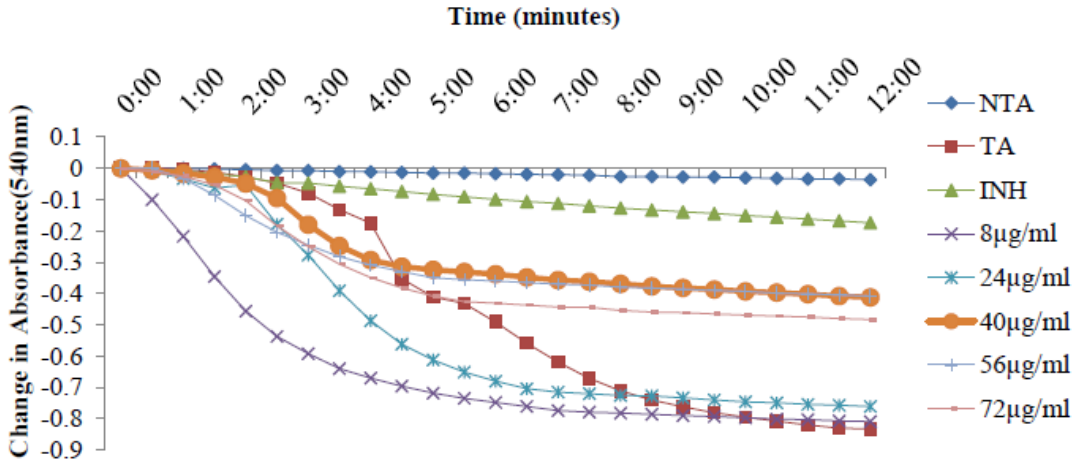

Figure 7:
Effect of varying concentrations of chloroform fraction of methanol extract of *A. conyzoides* (CFAC) on the mitochondrial permeability transition pore in the presence of calcium

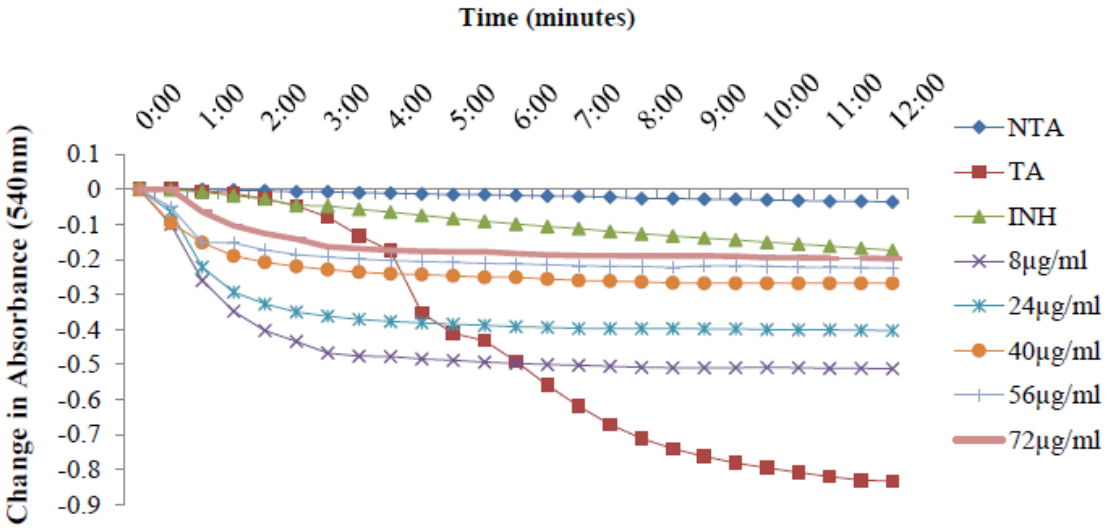

Figure 8:
Effects of varying concentrations of ethyl acetate fraction of *A. conyzoides* (EFAC) on mitochondrial permeability transition pore in the presence of calcium

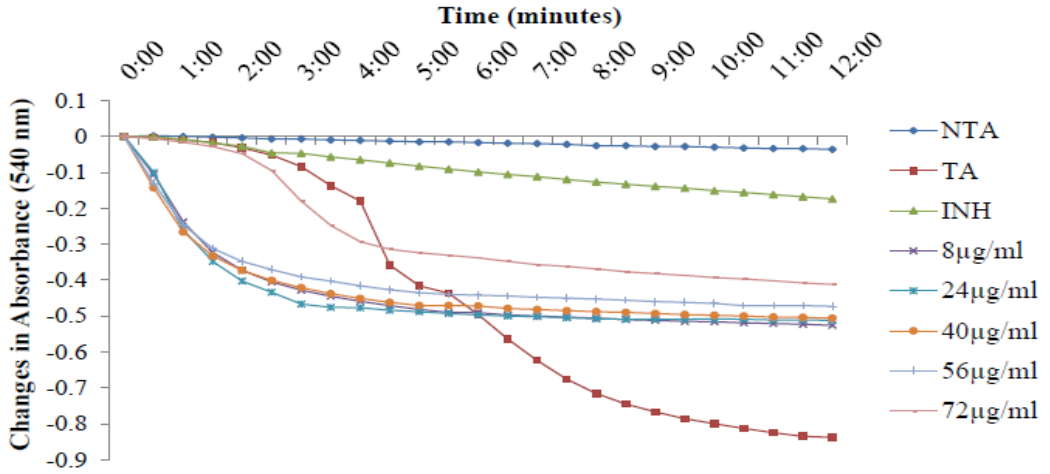

Figure 9:
Effect of varying concentrations of methanol fraction of methanol fraction of *A. conyzoides* (MFAC) on the mitochondrial permeability transition pore in the presence of calcium

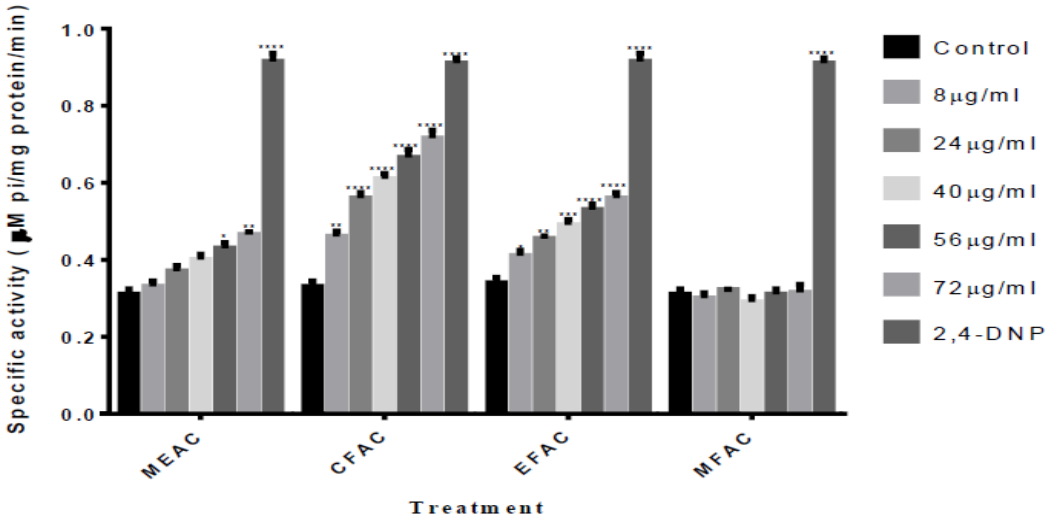

Figure 10:
Effects of different fractions of *Ageratum conyzoides* on specific activity of rat liver mitochondrial F_0F_1 ATPase
* p< 0.05, ** p < 0.01, *** p < 0.001, ****p < 0.0001 compared to the control.

Effect of various fractions of *Ageratum conyzoides* on mitochondrial ATPase activity

Figure 10 represents the effect of *Ageratum conyzoides* on mitochondrial F_0F_1 ATPase activity. The data obtained show that MEAC, CFAC and EFAC significantly ($P<0.05$) stimulated the mitochondrial F_0F_1 ATPase activity to varying degrees with CFAC having the highest effect while MFAC had no significant effect.

Effect of various fractions of *Ageratum conyzoides* on cytochrome c release: The effect *Ageratum conyzoides* on cytochrome c release is shown in Figure 11. As revealed from the results, there was significant release of cytochrome c in the order CFAC > EFAC > MEAC > MFAC.

Effect of various fractions of *Ageratum conyzoides* on mitochondrial lipid peroxidation: The effect of *Ageratum conyzoides* on ferrous-induced lipid peroxidation is shown in Figure 12. The results show that MEAC, CFAC, EFAC and MFAC all inhibited ferrous-induced lipid peroxidation. However, CFAC was the most potent with respect to the inhibition

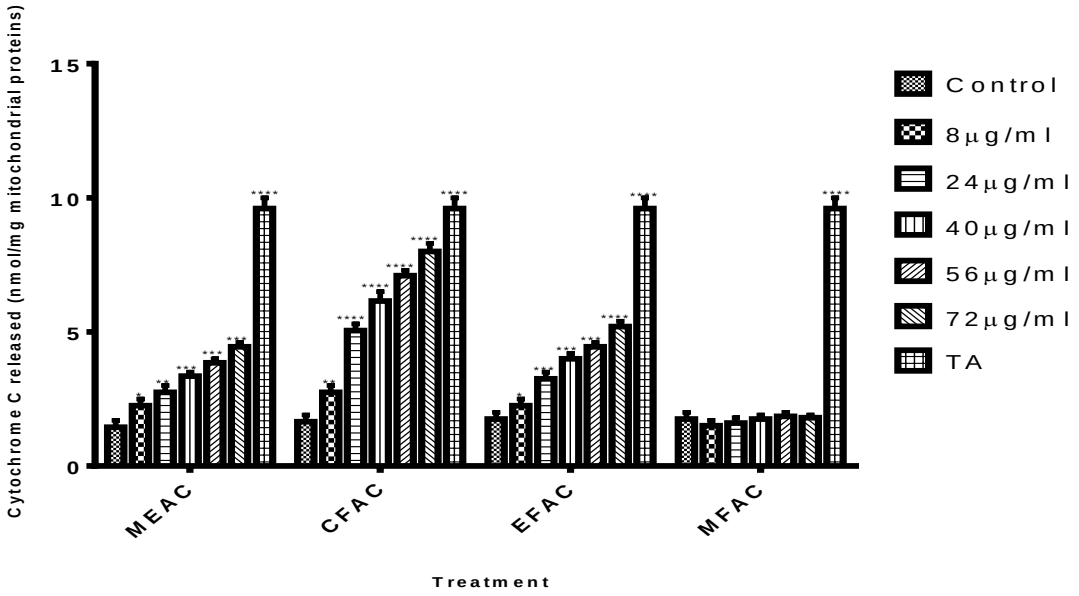

Figure 11: Effects of different fractions of *Ageratum conyzoides* on cytochrome c release
* $p< 0.05$, ** $p < 0.01$, *** $p < 0.001$, ****$p < 0.0001$ compared to the control

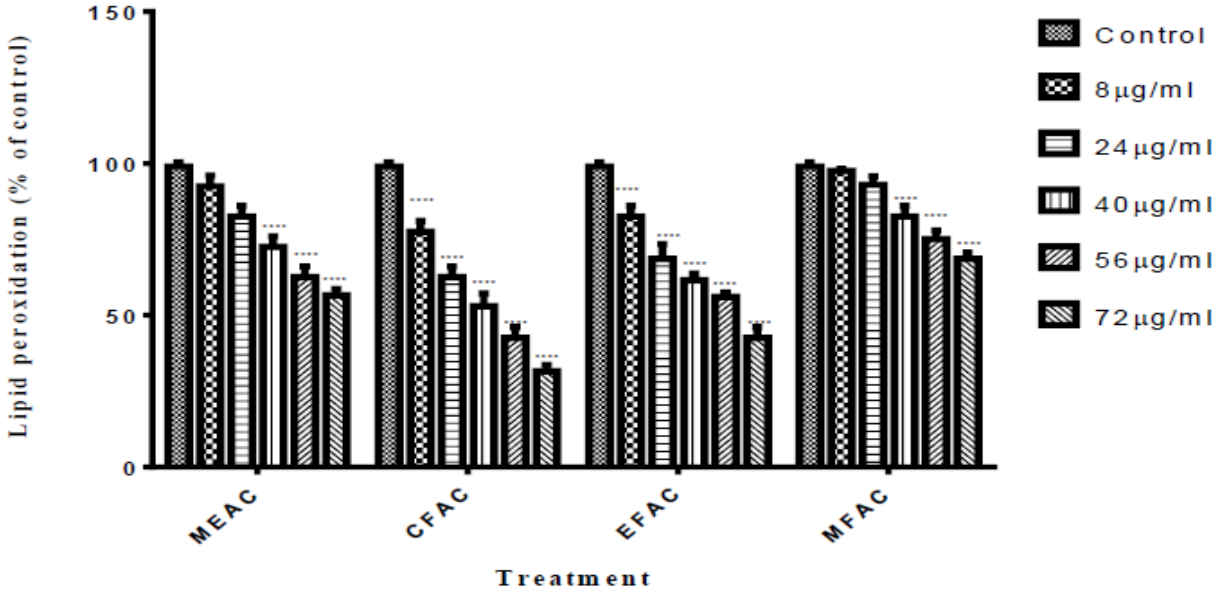

Figure 12:
Effects of different fractions of *Ageratum conyzoides* on lipid peroxidation
****$p < 0.0001$ compared to the control

DISCUSSION

Defect in apoptotic signaling pathway plays important role in malignant cell survival (Hanahan and Weinberg, 2000; Elmore, 2007; Lopez and Tait, 2015). Induction of apoptosis is one the most important markers of cytotoxic antitumor agents. In this study, the effects of various fractions of *Ageratum conyzoides* were investigated on mitochondrial-mediated apoptosis via induction of mPT pore opening. First, we determined the intactness and suitability of the mitochondria used for this study (Lapidus and Sokolove,1993; Javadov and Karmazyn, 2007). The opening of the mPT pore on exposure of the intact mitochondria to MEAC, MFAC, EFAC and CFAC (in the absence of calcium) suggests the presence of some phytochemicals in the fractions that could possibly interact with some mitochondrial components and thereby cause the opening of the pore. Based on their potency with respect to mPT pore opening, this study showed that the active component is highly present in the CFAC. However, reversal of calcium-induced pore opening by all the fractions suggests their calcium-chelating ability, thereby decreasing the calcium concentration available to induce mPT pore opening. This is similar to one of our previous findings where it was reported that the fractions of *Drymaria cordata* induced mPT pore opening in the absence of calcium, however, in the presence, there was reversal of calcium-induced pore opening (Olowofolahan et al., 2015). Studies have shown that the opening of the pore causes the release of cytochrome c from the mitochondrial inter membrane space to the cytosol. The opening of the pore by the fractions in this study caused significant release of cytochrome c with CFAC being the most potent, followed by EFAC and MEAC. This is also in accord with the mPT results where CFAC showed the highest inductive effect.

Opening of the mPT pore has also been shown to cause ATP hydrolysis (Kroemer, 2000; Halestrap and Pasdois, 2009; Jasiel et al. 2021). The release of inorganic phosphate was used as an index to measure the mitochondrial ATPase activity. The effect of the fractions was investigated on mitochondrial F_0F_1 ATPase activity and CFAC was found to be the most potent, followed by EFAC and MEAC.

Interestingly, the ability of the fractions to inhibit ferrous-induced lipid peroxidation could be possibly related to the presence of phenolic compounds, which accounts for the antioxidant activity of natural plant products and which have been shown to be free radical terminators (Nijveldt, 2001; Tohidi et al. 2017; Romelle et al. 2020). This also suggests their protective role on membrane bilayers by shielding the mitochondrial membranes from free radical-induced cellular damage. Moreover, it suggests that the mechanism of induction of pore opening is not via generation of reactive oxygen species that could lead to peroxidation of mitochondrial membrane lipids but rather via interaction with components of the pore. Although, this is surprising, but could be correlated with the findings of Adebayo et al. (2010), who demonstrated the anticancer and antiradical scavenging activity of *Ageratum conyzoides* and Adetuyi et al. (2018), who also reported the anti-lipidperoxidative effect of aqueous and methanol extracts of *Ageratum conyzoides* in the penile tissue of rats.

The results from this study suggest the presence of certain phytochemicals in the fractions of *Ageratum conyzoides* that could cause mPT pore opening, cytochrome c release, mitochondrial ATPase activity and possibly, cell death. The anti-lipidperoxidative potential suggests the presence of certain phytochemicals in the fractions with antioxidant property. However, the active principle is highly present in the non-polar chloroform fraction of the methanol extract; therefore, further studies are warranted to investigate its usefulness.

REFERENCES

Acheampong, F., Larbie, C., Fareed, K.N. (2015). Appiah-Opong R, Tuffour I. Antioxidant and anticancer study of Ageratum conyzoides Aqueous extracts, Journal of Global Biosciences, 4 (1): 1805-1815.

Adebayo, A.H., Tan, N.H., Akindahunsi, A.A., Zeng, G.Z., Zhang, Y.M. (2010). Anticancer and anti radical scavenging activity of Ageratum conyzoides, Pharmacogn Mag, 6(21): 62–66.

Adetuyi, F.O., Karigidi, K.O., Akintimehin, E.S., Adeyemo, N.O. (2018). Antioxidant properties of Ageratum conyzoides L. Asteraceae leaves, Bangladesh J. Sci. Ind. Res, 53(4): 265-276.

Bassir, O. (1963). Improving the level of nutrition, West African Journal of Biology and Applied Chemistry, 7:32-40.

Chan, W.H., Wu, H.J., Hsuuw, Y.D. (2005). "Curcumin inhibits ROS formation and apoptosis in methylglyoxal-treated human hepatoma G2 cells," Annals of the New York Academy of Sciences, 1042:372–378.

Danial, N.N., Korsmeyer, S.J. (2004). Cell death: critical control points, Cell, 116:205–19.

Du, C., Fang, M., Li, Y., Li, L., Wang, X. (2000). Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition, Cell, 102 : 33-42

Elmore, S. (2007). Apoptosis: A review of programmed cell death. Toxicol Pathol, 35(4):495–516.

Gibellini, L., Moro, L. (2021). Programmed Cell Death in Health and Disease. Cells, 2021, 10, 1765.

Gibellini, L., Pinti, M., Nasi, M. et al. (2011). "Quercetin and cancer chemoprevention." Evidence-Based Complementary and Alternative Medicine. Article ID 591356.

Goldar, Samira, Mahmoud Shekari , Khaniani Mahmoud, Shekari Khaniani, Sima Mansoori, Derakhshan Sima, Mansoori Derakhshan. (2015). Molecular Mechanisms of Apoptosis and Roles in Cancer Development and Treatment. Asian Pacific journal of cancer prevention, 16(6):2129-2144.

Green, D.R., Llambi, F. (2015). Cell death signaling. Cold Spring Harb Perspect Biol 7:006080.

Guimarães, C.A., Linden, A.R. (2004). Programmed cell deaths. European Journal of Biochemistry. 271(9):1638-50.

Gulbins, E., Dreschers, S., Bock, J. (2011). Role of mitochondria in apoptosis experimental physiology. 2011p. 85-90.

Halestrap, A. P. and Pasdois, P. (2009). The role of the mitochondrial permeability transition pore in heart disease. Biochim. Biophys. Acta 1787, 1402–1415.

Hanahan, D. and Weinberg, R. (2000). The hallmarks of cancer, Cell, 100:57–70.

Jasiel, O., Strubbe-Rivera, Jason, R. Schrad, Evgeny, V. Pavlov, James F., Conway, Kristin N., Parent and Jason, N. Bazil. (2021). The mitochondrial permeability transition

phenomenon elucidated by cryo-EM reveals the genuine impact of calcium overload on mitochondrial structure and function. Scientific Reports. 11: 1037.

Javadov, S., Karmazyn, M. (2007). Mitochondrial permeability transition pore as end point to cell death and as a putative target for cardioprotection, Cell Physio Biochem, 20: 1-22.

Johnson, D. and Lardy, H. (1957). Isolation of liver or kidney mitochondria, Methods Enzymol, 10:94-96.

Katewa, S.S.and Arora, A.(1997). Some plants in folk medicines of Udaipur district (Rajasthan), Ethnobot, 9:48-51.

Kroemer, G. and Reed, J.C. (2000). Mitochondrial control of cell death. Nat. Med, 6: 513–519.

Lapidus, R.G. and Sokolove, P.M. (1993). Inhibition by spermine of the inner membrane permeability transition of isolated heart mitochondria, FEBS. Lett, (3): 314-318.

Lockshin, R.A. and Zakeri, Z. (2004). Caspase-independent cell death?, Oncogene 23(16):2766-73.

Lopez, J., and Tait, S. W. G. (2015). Mitochondrial apoptosis: killing cancer using the enemy within. Br. J. Cancer. 112, 957–962.

Lowry, O.H., Rosenbrough, N.J., Farr, A. l., Randall, R.J. (1951). Protein measurement with Folin phenol reagent, J Biol Chem, 193:265-275.

Ming, L.C. (1999). In: J. Janick (ed.), Perspectives on new crops and new uses. Ageratum conyzoides: A tropical source of medicinal and agricultural products. p. 469–473 ASHS Press, Alexandria, VA 1999.

Nicholson, D.W. and Thornberry, N.A. (2003). Life and death decisions, Science, 299:214–215.

Niloufar karami, Ameneh Javid, Bibi Fatemeh Haghirosadat. (2017). A Review of Medicinal Plants Effective in the Treatment or Apoptosis of Cancer Cells. Cancer Press. 3(1): 22-26.

Nijveldt, R. (2001). Flavonoids : a review of probable mechanism of action and potential applications. Am J Clin Nutr, 74, 418–425.

Olorunsogo, O.O. and Malomo, S.O. (1985). Sensitivity of Oligomycin-inhibited respiration of isolated rat liver mitochondria to perfluidone, a fluorinated arylalkylsulfonamide. Toxicology. 1985; 35(3):231-40.

Olorunsogo, O.O., Bababunmi, E.A., Bassir, O. (1979). Uncoupling effect of Nphosphonomethylglycine on at liver mitochondria, Biochem. Pharm, 27: 925-927.

Olowofolahan, A.O., Adeoye, A.O., Offor, G.N., Adebisi, L.A., Olorunsogo, O.O. (2015). Induction of Mitochondrial Membrane Permeability Transition Pore and Cytohrome c Release by Different Fractions of Drymaria cordata, Archives of Basic and Applied Medicine, 3: 135-144.

Olowofolahan, A.O., Nwaokolo, E.O., Olorunsogo, O.O. (2019). Effect of Methanol Leaf Extract of Blighia Sapida on Mitochondrial Membrane Permeability Transition Pore Opening and Blood Glucose Levels in Normal and Streptozotocin-Induced Diabetic Rats, Biomed J Sci & Tech Res,19(1): 14047-14057.

Özlem Sultan Aslantürk. (2017). *In Vitro* Cytotoxicity and Cell Viability Assays: Principles, Advantages, and Disadvantages, Genotoxicity - A Predictable Risk to Our Actual World, Marcelo, L Larramendy and Sonia Soloneski, IntechOpen, DOI: 10.5772/intechopen.71923.

Rajwar, G.S. (1983). Low altitude medicinal plants of south Garhwal (Garhwal Himalaya). Bull Med. Ethnobot. Res, 1983; 4:14-28

Reed, J. (2004). Apoptosis mechanisms: implications for cancer drug discovery, Oncology. 2004; 18:11–20.

Romelle, F.D., Emmanuel, P.A., Ashwini, R.P., Quentin, M.T. and Carl Moses, M.F. (2020). "Effect of microwave blanching on antioxidant activity, phenolic compounds and browning behaviour of some fruit peelings," Food Chemistry, 302, Article ID 125308.

Rosangkima, G., Prasad, S.B. (2001). Antitumour activity of some plants from Meghalaya and Mizoram against Murineascites Dalton´s lymphoma. Ind. J. Exp. Biol, 192(10): 981-988.

Ruberto, G., Baratta, M.T., Deans, S.G., Dorman, H.J. (2000). Antioxidant and antimicrobial activity of Foeniculum vulgare and Crithmum maritimum essential oils. Planta Med, 66, 687.

Tohidi, B., Rahimmalek, M., and Arzani, A. (2017). Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of Thymus species collected from different regions of Iran. Food Chem. 220, 153–161.

Ungvari, Z., Labinskyy, N., Mukhopadhyay, P. et al. (2009). "Resveratrol attenuates mitochondrial oxidative stress in coronary arterial endothelial cells," The American Journal of Physiology. Heart and Circulatory Physiology, 297 (5):1876–1881.

Wijayakusuma, H., Dalimarta, S. (1994). Traditional concoction for cancer healing.. Penebar Swadaya, Jakarta. 64-65.

Yan, G., Elbadawi, M., Efferth, T. (2020). Multiple cell death modalities and their key features (Review). World Acad Sci J 2:39–48.