



Research Article

GCMS analysis of partially purified chloroform sub fractions of methanol extract of *drymaria cordata* (Linn) and their effects on mitochondrial membrane permeability transition pore

Olowofolahan A.O., Oyebode O.T., Olorunsogo O.O

Laboratories for Biomembrane Research and Biotechnology, Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Received: May 2019; Accepted: December, 2019

Abstract

The plant *drymaria cordata* is folklorically used in the treatment of uterine fibroids. Investigating its phytoconstituents and their effects on mitochondrial permeability transition (mPT) pore is of pharmacological importance. The aim of this study was to identify the possible phytochemical compounds present in the most potent sub-fraction of the plant and test them on mPT pore. The methanol extract of *drymaria cordata* was successively partitioned between n-hexane, chloroform, ethyl acetate and methanol solvent using Vacuum Liquid Chromatography (VLC) technique to obtain n-hexane, chloroform, ethyl acetate and methanol fractions. The chloroform fraction was partitioned into five different subfractions using VLC. The subfractions were tested for their potency in the induction of mPT pore opening. The subfraction D which was the most potent with respect to mPT pore opening was loaded on a column and eluted with n-hexane and chloroform varying the solvent systems in order of increasing polarity. The flow rate was 2.5ml/min. Thin Layer Chromatography (TLC) plates were used to monitor samples with similar components. Samples with similar R_f values were pooled together. For further resolution, samples which showed the same single band on the TLC plates were further subjected to purification using micro-column chromatography. The eluents were also monitored using the TLC plates. The partially purified samples were subjected to GC-MS analysis and certain phytochemicals including hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, hexadecanoic acid, 9-Octadecenoic acid, Oxalic acid 2-ethylhexyl isobutyl ester, 3-(p-Fluorobenzoyl)-propionic acid and Bis(2-ethylhexyl) phthalate were identified. The presence of these major compounds might be responsible for the pharmacological properties ascribed to the fraction. Also, these could give an insight into developing chemopreventive and therapeutic approaches to various diseases in human associated with dysregulated apoptosis.

Key Words: *Drymaria cordata*, Mitochondrial membrane permeability transition pore, Gas Chromatography, Thin Layer Chromatography, Apoptosis.

INTRODUCTION

Phytochemicals are secondary metabolites that are naturally found in plants with several roles to play. These types of products could be phenolic compounds, carotenoids, products with nitrogen, alkaloids, fatty acids, organosulfur compounds, etc (Han, 2007 : Tsao *et al.*, 2010). Every class of phytochemicals has its biological active effect (Russo *et al.*, 2010). Natural compounds have been associated with antioxidant properties and prevention of free radicals generation. Recently, studies have shown that natural compounds have a more complex protective action at cellular and molecular levels, with important application in disease prevention or treatment (Braicu *et al.*, 2011).

The re-emergence of natural products in the management and treatment of diseases is as a result of increased number of

publications which focused on a better comprehension of their biological and beneficial properties in human health. Phytochemicals are now proved to play active role in chemoprevention or chemotherapy (Smith *et al.*, 2011; Baker *et al.*, 2017). Natural phytochemicals have been used in the prevention and treatment of cancer in folkloric medicine because of they are safe and readily available, from a wide range of natural sources. The plant-based natural constituents can be gotten from any part of the plant. Screening active compounds from plants has lead to the invention of new medicinal drugs which have different therapeutic effect against various diseases. Identification and quantification of these plant-based constituents may be relevant in the formulation of herbal for management and treatment of diseases. *Drymaria cordata* (Linn.) Willd. (Caryophyllaceae) is a creeping herb growing in dense patches in moist shady

places and also in dry sun-exposed areas. The plant is found widely dispersed in damp places all over the tropics of Africa, Asia and the Americas where its various uses for agriculture and traditional medicine have been reported (Telefo, *et al.*, 2011 ; (Adeyemi *et al.*, 2008). The anti-inflammatory (Mukherjee *et al.*, 1998), antitussive (Mukherjee *et al.*, 1997), antibacterial (Mukherjee *et al.*, 1998), cytotoxic (Sowemimo *et al.*, 2009), anxiolytic (Barua *et al.*, 2009) activity, analgesic, anti-nociceptive and antipyretic properties (Akindele *et al.*, 2012; Barua, *et al.*, 2011; Barua, *et al.*, 2009) of *Drymaria cordata* extract have been reported Previous studies in our laboratory have shown that chloroform fraction of methanol extract of *drymaria cordata* (CFDC) is highly potent in the induction of mitochondrial-mediated apoptosis (Olowofolahan *et al.*, 2015; Olowofolahan *et al.*, 2018), reversal of MSG-induced cellular injury and MSG-induced uterine hyperplasia (Olowofolahan *et al.*, 2017). The phytochemical constituents of CFDC that is responsible for these pharmacological properties have not yet been explored. Gas Chromatography-Mass Spectrum (GCMS) analysis was thus employed for this study.

MATERIALS AND METHODS

Collection of fresh *Drymaria cordata*: The whole plant of *Drymaria cordata* were freshly harvested and obtained from Department of Botany, University of Ibadan, Nigeria. Samples were authenticated and identified at the Herbarium, Department of Botany, University of Ibadan, Ibadan, Oyo State and a specimen Voucher No.UIH-22555 was deposited in the Herbarium. The plants were washed, air-dried for three weeks in the laboratory after which they were powdered with industrial machine and weighed.

Preparation of Crude Methanol Extract and Chloroform Fraction of *Drymaria cordata*: 6-kilogramme air-dried, whole plant of *Drymaria cordata* were extracted with sufficient methanol (Sigma Aldrich Chemical Co. St Louis USA) 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 (Stuart) to yield a dark residual solution. This solution is made up of two main layers, an oily upper phase and a brown residue and these two layers were separated by decantation . The crude methanol extract was heated over a water bath at 40°C to obtain a solvent free extract. The crude methanol extract was further partitioned between n-hexane, chloroform, ethylacetate and methanol using vacuum liquid chromatography (VLC) technique. All these fractions were concentrated to dryness under pressure using rotary evaporator at 40°C to obtain the n-hexane (HFDC), chloroform (CFDC), ethylacetate (EFDC) and the methanol (MFDC) fractions.

Experimental Animals: Male Wister strain albino rats weighing between 100g–120g were purchased from the Preclinical Animal House of the College of medicine, University of Ibadan, Ibadan, Nigeria. All the animals were allowed two weeks period of acclimatization in the Animal House of the Department of Biochemistry, University of Ibadan. The animals were placed under a 12hr light/dark cycle and fed commercial pelletized rat chow and water *ad libitum* throughout the experimental period. All experiments have

been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Isolation of Rat Liver Mitochondria: Rat liver mitochondria were isolated essentially according to the method of Johnson and Lardy, (1967) and as modified by Olorunsogo and Malomo, (1989).

Mitochondrial Swelling Assay: Mitochondrial membrane permeability transition was monitored by measuring changes in absorbance of mitochondrial suspension in a T70 UV visible spectrophotometer essentially according to the method of Lapidus and Sokolove (1993).

Determination of mitochondrial protein: Mitochondrial protein concentration was determined according to the method of Lowry *et al.*, (1951). using bovine serum albumin as standard.

Vacuum Liquid Chromatography of the chloroform fraction

Packing of the Chromatographic column The prewashed sintered glass Buchner was further cleaned with concentrated H₂SO₄ to remove impurities from the sieve. The column was then packed three-quarters full with silica gel 60 (0.040–0.063mm, MERCK). The column was then placed on a conical Buchner flask and connected to the vacuum pump. The pump was switched on and n-hexane solvent was applied to the column. This was done to further pack the column.

Preparation of the Sample Slurry: Silica gel 60 (0.040–0.063mm, MERCK) 8g was added to 12g of the chloroform fraction sample. The gel-sample mixture was stirred until a homogenous mixture was obtained. The mixture was air-dried to obtain a powder form.

Loading of sample on the column: The sample was applied to the top of the column with the pump switched on, the first solvent system, 100% n-hexane was added to the column. The flow rate was 25ml/min. This was done with 1000ml of the n-hexane solvent. The column was eluted again with n-hexane : chloroform (1:4) , (1:1) and (4:1). This was done until there was a complete exhaustion of the fraction in the column. The column was further eluted with chloroform only (100%) and lastly with chloroform: methanol (4:1). The order of elution is as follows:

n-hexane	100%	(A)
n-hexane:chloroform	80:20	(B)
n-hexane:chloroform	50:50	(C)
n-hexane:chloroform	20:80 *	(D)
Chloroform	100%	(E)
Chloroform:methanol	80:20	(F)

The fractions obtained were concentrated at 40°C using rotary evaporator and transferred into pre-weighed all-glass sample bottles with rubber stoppers and labeled. The most potent with respect to induction of mPT pore opening was subjected to column chromatography and thin layer chromatography.

The most potent sub fraction (D) with respect to induction of pore opening was adsorbed on silica gel 60 (0.040–0.063mm, MERCK) and applied to a normal column packed with silica gel using n-hexane. Thin Layer Chromatography (TLC) of the sub-fractions were carried out in order to assess the purity and

also to identify the phytochemicals present in each of the sub-fractions gotten from the solvent systems. The column was eluted using the solvent systems shown below. The flow rate was 2.5ml/min.

- n-hexane 100%
- n-hexane :chloroform 95:5
- n-hexane :chloroform 90:10 * tubes 21-35 (G) *
- n-hexane :chloroform 85:15
- n-hexane :chloroform 80:20
- n-hexane :chloroform 75:25 * tubes 116-136 (H) *
- n-hexane :chloroform 70:30
- n-hexane :chloroform 65:35 * tubes 152-183 (I) *
- n-hexane :chloroform 60:40

Fractions that showed similar components on TLC were pulled together. The samples G, H and I which showed single band on the TLC plates were further subjected to micro-column chromatography for a better resolution and more purified sample using the same solvent system

Sample G - tubes 10 & 11

Sample H - tubes 10, 11, 12 and 13

Sample I - tubes 13 & 14

The samples obtained were subjected to Gas Chromatography Mass Spectrum (GCMS).

GC-MS analysis of samples G, H and I partially purified from chloroform fraction of methanol extract of *drymaria cordata*

The chloroform fraction of methanol extract of *drymaria cordata* was subjected to GC-MS analysis using Agilent technologies 7890 GC system and the model of the detector is Agilent technologies 5975 MSD (Mass Spect. Detector). The principle behind the GC analysis is separation techniques. In separation techniques, there are two phases – the mobile and the stationary phase. The mobile phase is the carrier gas (Helium, 99.99% purity), while the stationary phase is the column. The model of the column is HP5 MS with length 30 m, internal diameter 0.320 mm, while the thickness is 0.25 µm. The oven temperature program is initial temperature of 80°C to

hold for 1 minute. It increases by 10°C per minute to the final temperature of 240°C to hold for 6 minutes. The injection volume is 1 microlitre and the heater or detector temperature is 250°C.

The sample extracted is put in a vial bottle and the vial bottle is placed in auto injector sample compartment. The automatic injector injects the sample into the liner. The mobile phase pushes the sample from the liner into the column where separation takes place into different components at different retention time. The MS interpret the spectrum MZ (mass to charge ratio) with molar mass and structures.

RESULTS

The figure 1a shows the chromatogram of sample G that was partially purified from chloroform fraction of methanol extract of *drymaria cordata*. Figures 1b-d depicts the various phytochemicals found in the sample with their respective percentage of abundance.

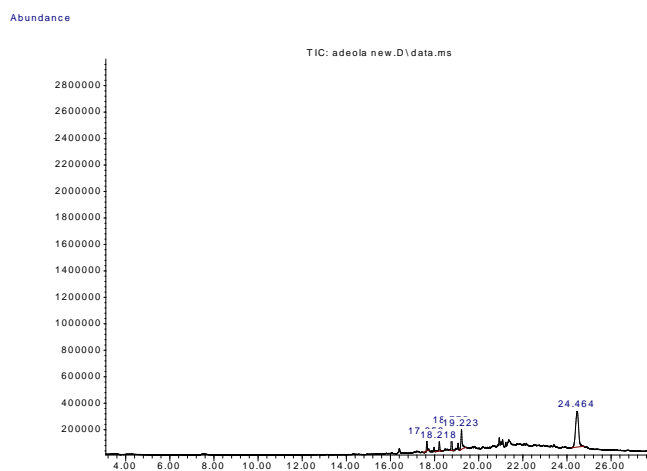


Figure 1a: GC-MS chromatogram of sample G partially purified from chloroform fraction of methanol extract of *Drymaria cordata*

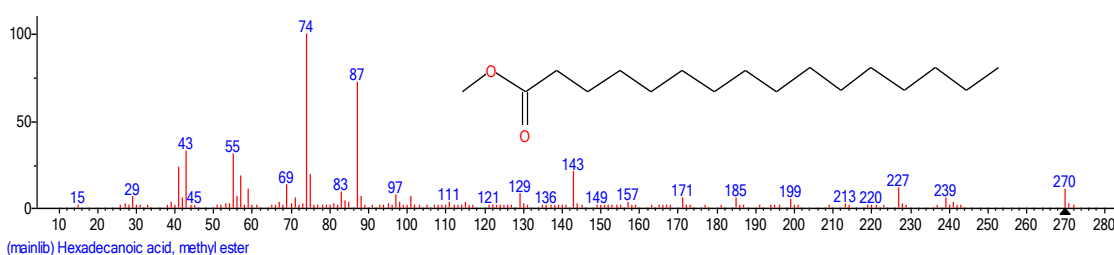


Figure 1b: Mass spectra of the peak having retention time 18.775 (11.297%)

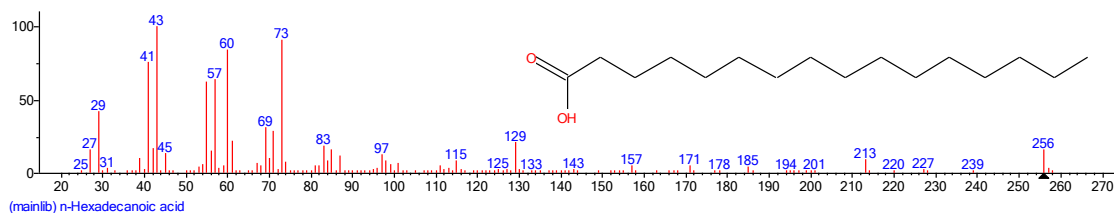


Figure 1c: Mass spectra of the peak having retention time 19.223 (14.771%)

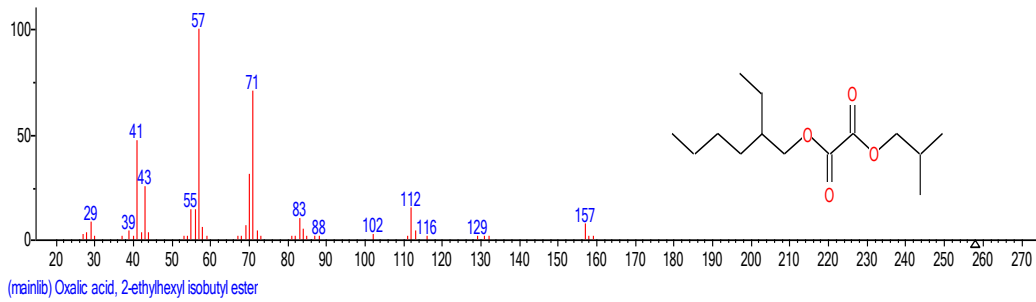


Figure 1d: Mass spectra of the peak having retention time 24.464 (63.660%)

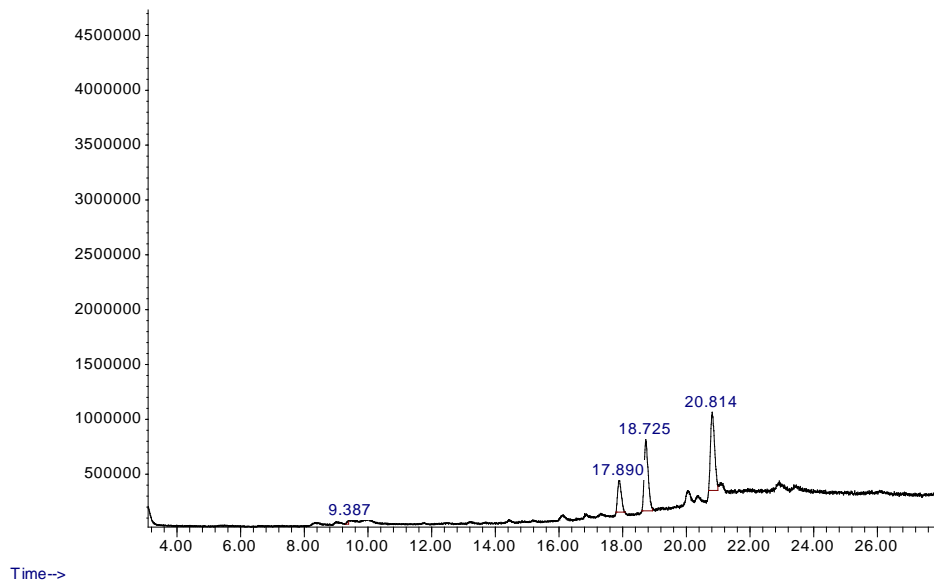


Figure 2a: GC-MS chromatogram of sample H partially purified from chloroform fraction of methanol extract of *Drymaria cordata*

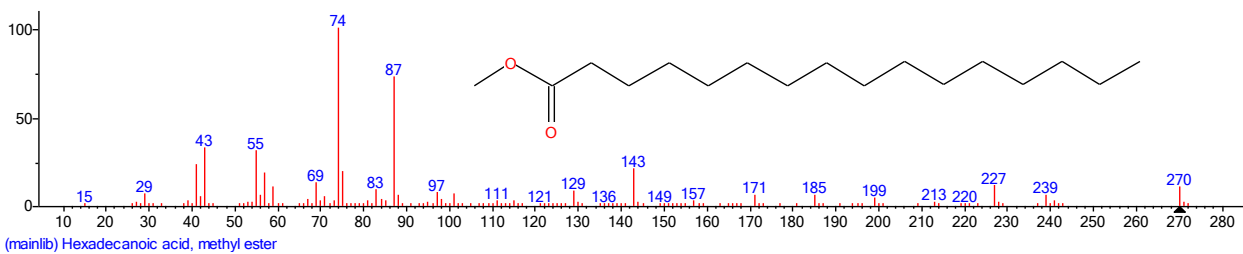


Figure 2b: Mass spectra of the peak having retention time 17.890 (16.11%)

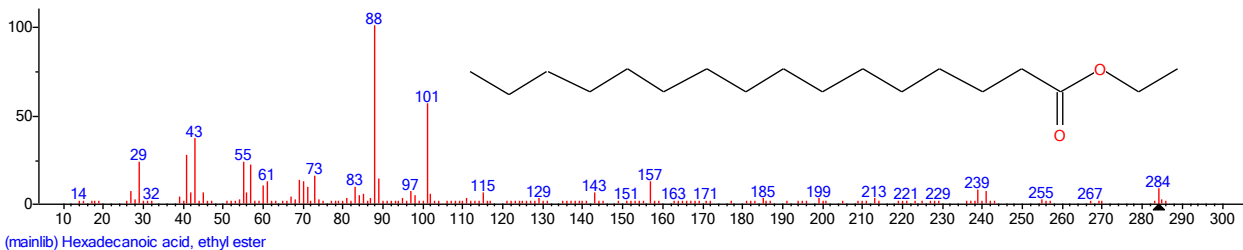


Figure 2c: Mass spectra of the peak having retention time 18.725 (38.98%)

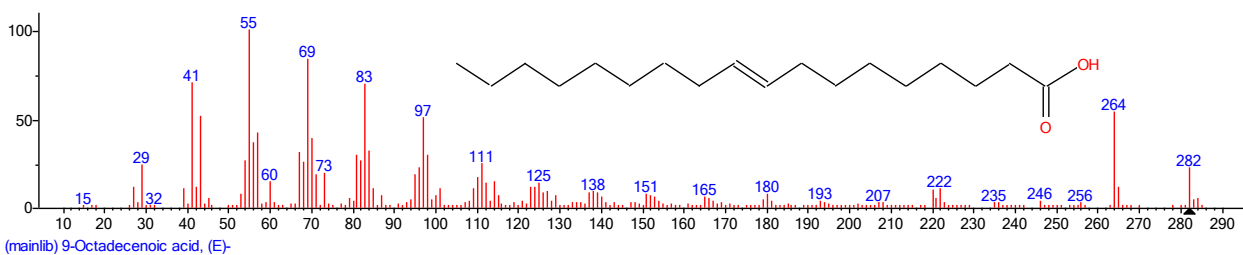


Figure 2d: Mass spectra of the peak having retention time 20.814 (44.14%)

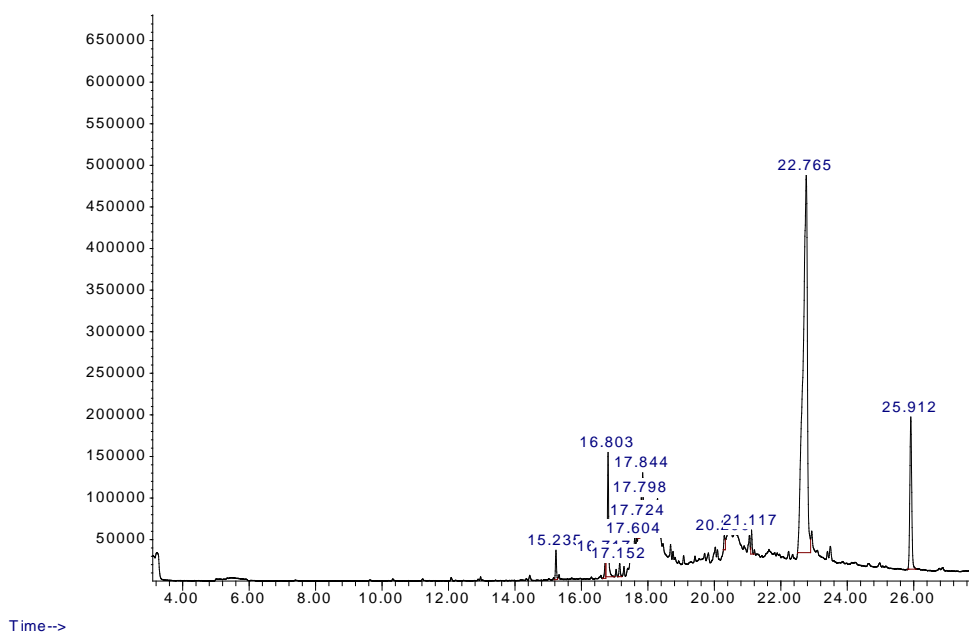


Figure 3a: GC-MS chromatogram of sample I partially purified from chloroform fraction of methanol extract of *Drymaria cordata*

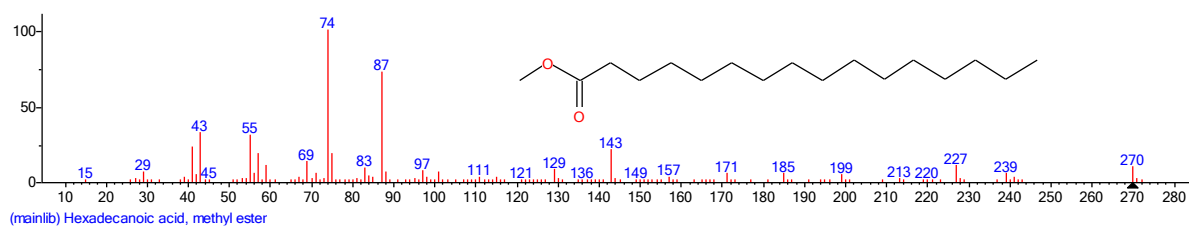


Figure 3b: Mass spectra of the peak having retention time 17.844 (2.350%)

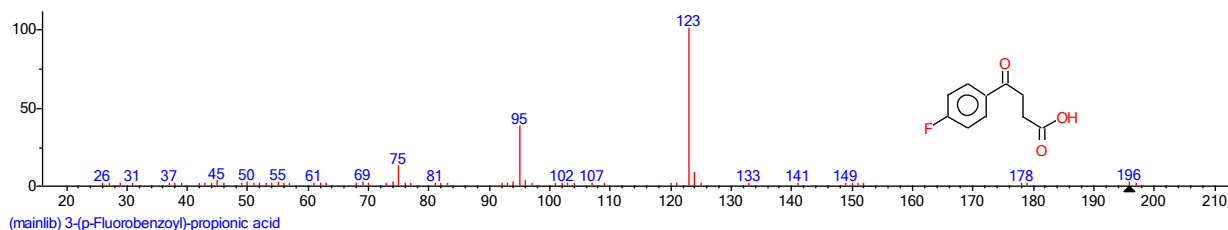


Figure 3c: Mass spectra of the peak having retention time 22.765 (70.235%)

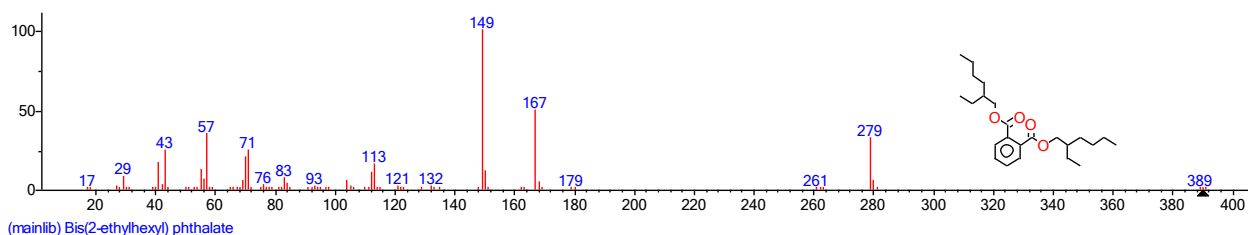


Figure 3d: Mass spectra of the peak having retention time 25.912 (11.964%)

Table 1a:

Compounds from partially purified sample G of chloroform fraction of methanol extract of *drymaria cordata* analysed through GC-MS analysis.

Name Of The Partially Purified Compounds In Sample G	%	Activity
Oxalic acid 2-ethylhexyl isobutyl ester	63.666%	No report
N-Hexadecanoic acid	14.711%	Nematicide, 5-Alpha-Reductase-Inhibitor, Flavor enhancer, Hypocholesterolemic Pesticide , Antialopecic ,Antiandrogenic, Antifibrinolytic, Antifungal and Antioxidant
N-hexadecanoic acid methyl ester	11.297%	anticancer, anti-microbial, antioxidant

Figure 3a shows the GC-MS chromatogram of sample I partially purified from chloroform fraction of methanol extract of *Drymaria cordata*. The phytochemicals present in the sample were highlighted in figures 3b-d

Tables 1a,b and c show the names, percentage abundance and the pharmacological activities of the various phytochemical compounds present in the partially purified samples from the chloroform fraction of methanol extract of *drymaria cordata*.

Figure 4 shows that mitochondria used in this study to investigate the effect of the identified compounds on mPT pore opening were intact and suitable for use. This is because there was no significant swelling of the mitochondria over a period of twelve minutes in the absence of calcium. Upon the addition of calcium which is a triggering agent, there was an amplitude swelling of the mitochondria as seen from the graph. The calcium-induced pore opening was significantly reversed by the addition of spermine, a standard inhibitor of mPT pore opening showing that the mitochondria were intact *al binitio*

Figure 5 shows the effect of two varying concentrations of sample G (which is very high in Oxalic acid 2-ethylhexyl isobutyl ester) on mPT pore opening. There was a concentration-dependent induction of pore opening when compared with the control.

Also, sample H (containing majorly 9-Octadecenoic acid, hexadecanoic acid methyl ester and hexadecanoic acid ethyl ester) caused significant induction of mPT pore opening when compared with the control as seen from figure 6.

The major phytoconstituent in sample I is 3-(p-Fluorobenzoyl)-propionic acid (70.23%). This also caused significant induction of pore opening compared with the control (Fig. 7).

Table 1b

Compounds from partially purified sample H of chloroform fraction of methanol extract of *drymaria cordata* analysed through GC-MS analysis.

Name Of The Partially Purified Compounds In Sample H	%	ACTIVITY
Hexadecanoic acid methyl ester	16.11%	As indicated in sample G
Hexadecanoic acid ethyl ester	(38.98%)	Antiinflammatory property
9-Octadecenoic acid	(44.14%)	Cancer preventive, Anemiagenic, Insectifuge, Antiandrogenic, Dermatitigenic.

Table 1 C

Compounds from partially purified sample I of chloroform fraction of methanol extract of *drymaria cordata* analysed through GC-MS analysis.

Name Of The Partially Purified Compounds In Sample I	%	Activity
3-(p-Fluorobenzoyl)-propionic acid	70.235%	Inhibitor of MAPK signaling
Bis(2-ethylhexyl) phthalate	11.964%	Used as plasticizer, hydraulic fluid, dielectric fluid, and solvent
Hexadecanoic acid methyl ester	2.350%	As indicated in sample G

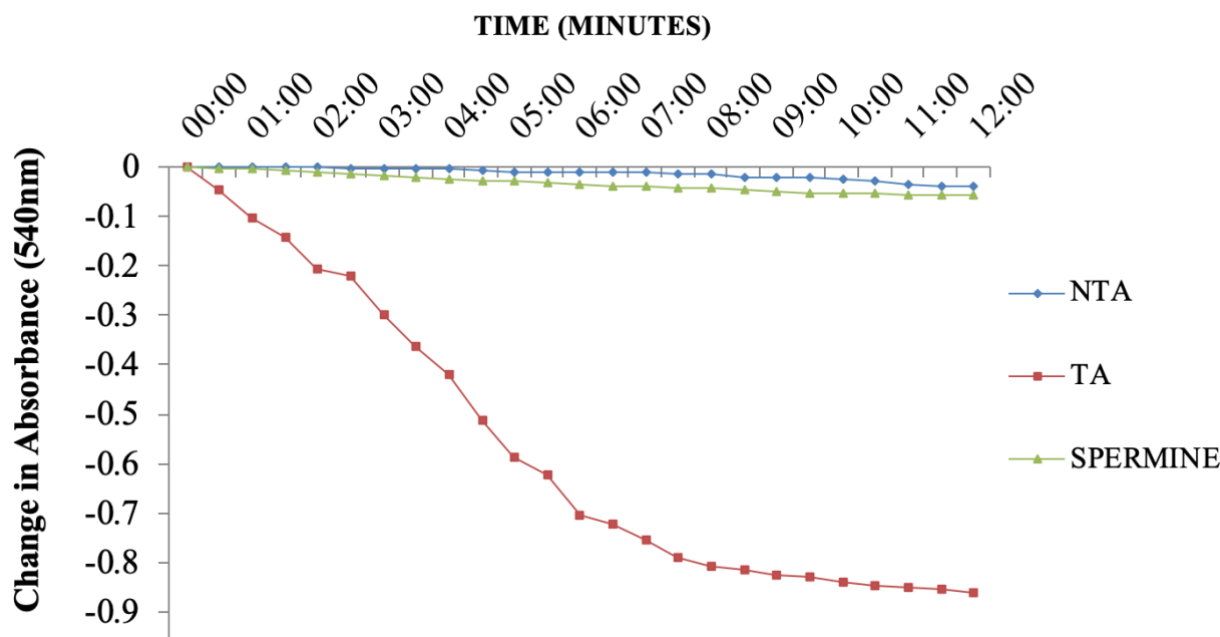


Figure 4: Determination of intactness of rat liver mitochondria
NTA: Non Triggerin Agent
TA: Triggering Agent

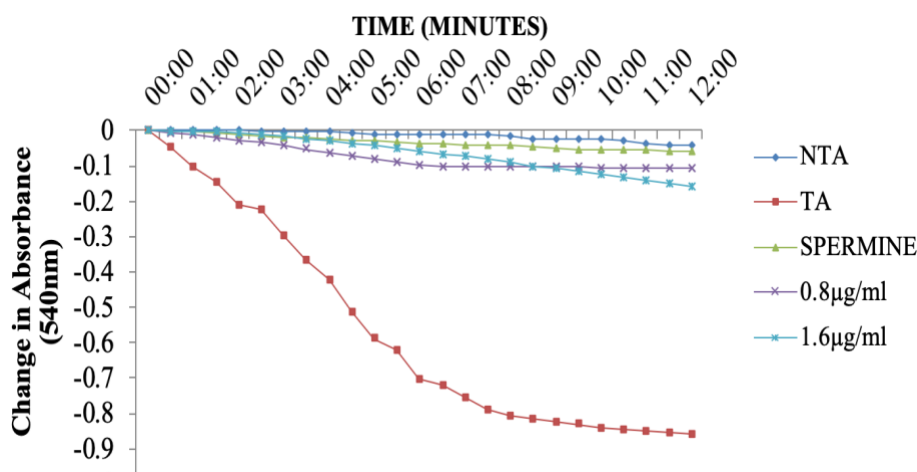


Figure 5:
Effect of partially purified sample G on rat liver mitochondrial permeability transition pore opening

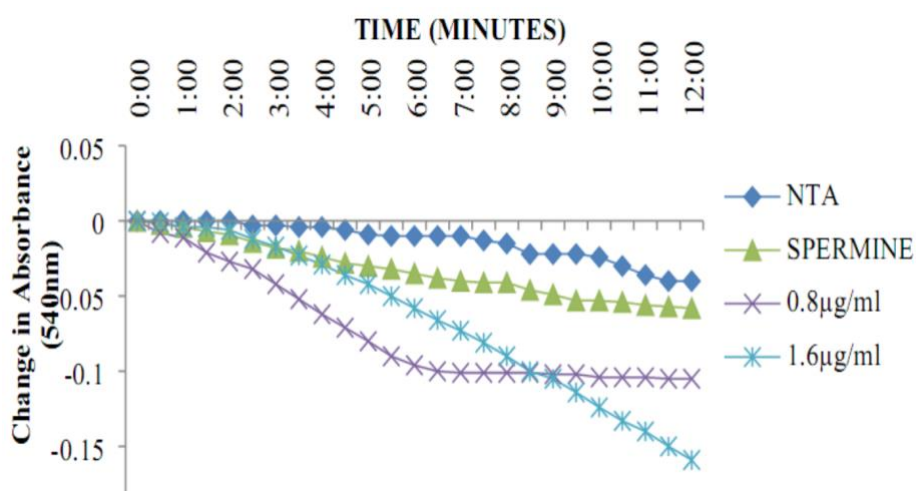


Figure 6:
Effect of partially purified sample H on rat liver mitochondrial permeability transition pore opening

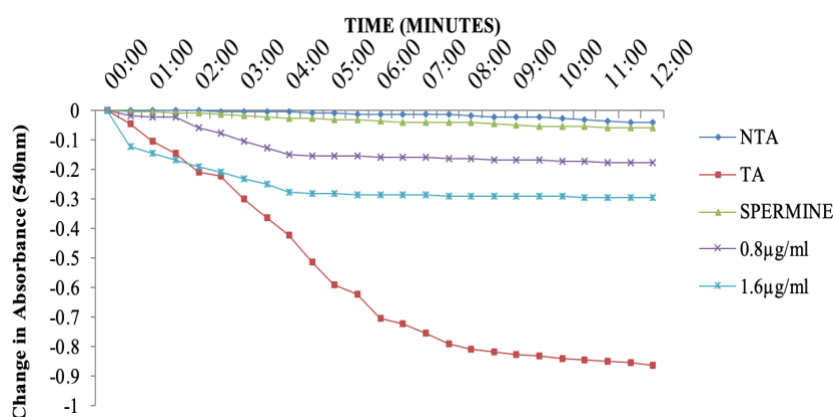


Figure 7:
Effect of partially purified sample I on rat liver mitochondrial permeability transition pore opening

DISCUSSION

The search for therapeutic agents from medicinal plants for various illness is really on the move and the chemopreventive and therapeutic potentials of a large number of herbs have been explored. *D. cordata* which belongs to the family of

caryophyllacea is one of such plant species that has been endowed with various pharmacological properties for the treatment of various ailments and also as folklore medicine. The choice of chloroform fraction of methanol extract of *drymaria cordata* for this study is based on our previous findings which showed the potency of the fraction in the

induction of mitochondrial-mediated apoptosis and amelioration of MSG-induced uterine hyperplasia in rats. In this study, the GC-MS analysis of partially purified samples from chloroform fraction of methanol extract of *drymaria cordata* revealed the presence of some phytochemicals including hexadecanoic acid, hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, Bis(2-ethylhexyl) phthalate, 3-(p-Fluorobenzoyl)-propionic acid, 9-Octadecenoic acid and Oxalic acid 2-ethylhexyl isobutyl ester as shown in Tables 1a-c.

The identified compounds have been reported to have various activities including cancer preventive, nematicide, 5-Alpha-Reductase-Inhibitor, flavour enhancing, hyporcholesterolemic, pesticide, antiallopecic, antiandrogenic, antifibrinolytic, antifungal, antioxidant, antimicrobial, antidiabetic, anemiagenic, insectifuge, antiandrogenic, dermatitogenic, antifungal, inhibitor of MAPK signaling. The anticancer and antioxidant properties are shown by 9-Octadecenoic acid and n hexadecanoic acid, methyl ester (Rajeswari *et al.*, 2012; Vijisara *et al.*, 2014; Gomathi *et al.*, 2015). The presence of these phytochemical compounds in the sample of partially purified chloroform fraction of methanol extract of *drymaria cordata* correlates with the anticancer property of the plant as it is used folklorically used in the treatment of uterine, liver, skin and breast tumour. Furthermore, the anticancer and antioxidant property is well demonstrated by the chloroform fraction of the plant being a potent inducer of mitochondrial-mediated apoptosis and inhibitor of lipid peroxidation (Olowofolahan *et al.*, 2015; Olowofolahan and Olorunsogo, 2018). Also, the 3-(p-Fluorobenzoyl)-propionic acid which is identified in sample I of the partially purified chloroform fraction is known to be an inhibitor of MAPK signaling pathway (Hyeon *et al.*, 2006). MAPK has been implicated in the development of uterine fibroid cell proliferation (Huang *et al.*, 2012). This implies that compound that inhibits MAPK signaling pathway could inhibit cellular proliferation. The presence of 3-(p-Fluorobenzoyl)-propionic acid in the chloroform fraction of methanol extract of *drymaria cordata* (as an inhibitor of MAPK) correlates with its ability to ameliorate MSG-induced uterine hyperplasia in rat (Olowofolahan *et al.*, 2017). Also, the presence of antioxidant phytochemicals like *n*-Hexadecanoic acid and *n*-Hexadecanoic acid, methyl ester might be the reason for its inhibitory effect on lipid peroxidation. The results on the effects of the partially purified samples G, H and I on mitochondrial membrane permeability transition pore opening revealed that they all induced mPT pore opening in a concentration-dependent manner. This is very relevant in diseased condition associated with dysregulated apoptosis. These also suggest that the partially purified fractions are potent inducers of mitochondrial-mediated apoptosis like the parent chloroform fraction. Furthermore, from the results, the degree of potency of the partially purified fractions in the induction of mPT pore opening show that purification enhances activity when compared with the potency of the parent chloroform fraction (Olowofolahan *et al.*, 2015). This study shows that CFDC contains active principles which might be relevant in combating several diseased conditions especially cancers. The various phytochemicals identified in this fraction could be an insight to developing chemopreventive and therapeutic approaches to various diseases in human.

REFERENCES

- Akindele, A.J. I F Ibe, and O O Adeyemi (2012) Analgesic and Antipyretic Activities of *Drymaria Cordata* (Linn.) Willd (Caryophyllaceae) Extract African Journal of Traditional, Complementary and Alternative Medicines 9(1):25-35 •
- Baker, M. (2017). Deceptive curcumin offers cautionary tale for chemists. *Nature*, 541, 144–145.
- Barua, C.C., Roy, J.D., Buragohain, B., Barua, A.G., Borah, P. and Lahkar, M. (2009) Anxiolytic Activity of Hydroethanolic Extract of *Drymaria cordata* Willd. *Indian Journal of Experimental Biology*, 47, 969-973.
- Barua, C.C., Roy, J.D., Buragohain, B., Barua, A.G., Borah, P. and Lahkar, M. (2011) Analgesic and Anti-Nociceptive Activity of Hydroethanolic Extract of *Drymaria cordata* Willd. *Indian Journal of Pharmacology*, 43, 121-125
- Braicu, E. I., J. Sehouli, R. Richter, K. Pietzner, C. Denkert, and C. Fotopoulou. 2011. Role of histological type on surgical outcome and survival following radical primary tumour debulking of epithelial ovarian, fallopian tube and peritoneal cancers. *British Journal of Cancer* 105:1818-1824.
- Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C (2015) GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides*(L.) L. *J Food Sci Technol* 52: 1212-1217.
- Han, X.; Shen, T.; Lou, (2007) H. Dietary polyphenols and their biological significance. *Int. J. Mol. Sci.*, 8, 950–988.
- Huang S, H.Izel M, Knijnenburg T, et al. 2012. MED12 controls the response to multiple cancer drugs through regulation of TGF-beta receptor signaling. *Cell* 2012; 151:937-50.
- Hyeon Soo Kim, Minseok Song, Sanatombi Yumkham, Jang Hyun Choi, Taehoon Lee, Joseph Kwon, Sung Jae Lee, Jong-In Kim, Kang Woo Lee, Pyung-Lim Han, Seung Woo Shin, Ja-Hyun Baik, Yong Sik Kim, Sung Ho Ryu and Pann-Ghill Suh (2006). Identification of a new functional target of haloperidol metabolite: implications for a receptor-independent role of 3-(4-fluorobenzoyl) propionic acid. *Journal of neurochemistry* 2006-10-13.
- Johnson D, Lardy H. (1967). Isolation of liver or kidney mitochondria. *Methods Enzymol.* 1967;10:94-96.
- Lapidus, R. & Sokolove, P. 1992. Inhibition by Spermine of the inner membrane permeability transition of isolated rat heart mitochondria. *FEBS Letter*, 3, 314–318.
- 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.
- Lowry, O.H., Rosebrough, N.J., Farr, A.I. and Randall, R.J. 1951. Protein measurements with the folin-phenol reagent. *J. Biol.Chem.*; 193:260-265
- Mukherjee PK, Mukherjee K, Das J, Pal M, Saha BP. 1997. Studies on the anti-inflammatory effects of *Drymaria cordata* Willd. *Natural Product Sciences* 63: 367- 69.
- Mukherjee, P.K., Bhattacharya, S., Saha, K., Giri, S.N., Pal, M. and Saha, B.P. 1998. Antibacterial Evaluation of *Drymaria cordata* Willd (Fam. Caryophyllaceae) Extract. *Phytotherapy Research*, 11, 249-250.
- Olorunsogo OO, Malomo SO. 1985. Sensitivity of Oligomycin inhibited respiration of isolated rat liver mitochondria to perfluidone, a fluorinated aryl alkyl sulfonamide. *Toxicology*. 35(3):231-40.

- Olowofolahan A.O and Olorunsogo O.O. 2018. Induction of apoptosis in rat liver cells via caspase activation by chloroform fraction of methanol extract of *drymaria cordata* 5 (4)
- Olowofolahan AO, Aina OO, Hassan ET, Olorunsogo OO. 2017. Ameliorative potentials of methanol extract and chloroform fraction of *Drymaria cordata* on MSG-induced uterine hyperplasia in female wistar rats. *European Journal of Medicinal Plants*. 20(4): 1-9
- Olowofolahan A.O, Adeoye O.A, Offor G.N, Adebisi L.A and Olorunsogo O.O. 2015. Induction of Mitochondrial Membrane Permeability Transition Pore Opening and Cytochrome C Release by Fractions of *Drymaria cordata*. *Arch. Bas. App. Med.*, 3; 135–144.
- Rajeswari G, Murugan M, Mohan VR 2012. GC-MS analysis of bioactive components of *Hugonia mystax* L (Linaceae). *Res J Pharmaceut Biol Chem Sci* 3: 301-308.
- Russo SJ, Dietz DM, Dumitriu D, Morrison JH, Malenka RC, Nestler EJ. 2010. The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends Neurosci*. 33:267–276.
- Smith, T.J. 2011. Green Tea Polyphenols in drug discovery— A success or failure? *Expert Opin. Drug Discov*, 6, 589–595.
- Sowemimo, A., Van de Venter, M., Baatjies, L. and Koekemoer, T. 2009. Cytotoxic Activity of Selected Nigerian Plants. *African Journal of Traditional Complementary and Alternative Medicine*, 6, 526-528.
- Telefo, P.B., Lienou, L.L., Yemele, M.D., Lemfack, M.C., Mouokeu, C., Goka, C.S., Tagne S.R. and Moundipa F.P. 2011. Ethnopharmacological Survey of Plants Used for the Treatment of Female Infertility in Baham, Cameroon. *Journal of Ethnopharmacology*, 136, 178-187.
- Tsao, R. 2010. Chemistry and biochemistry of dietary polyphenols. *Nutrients*, 2, 1231–1246.
- Vijisara ED, Subramanian A. 2014. GC-MS analysis of ethanol extract of *Cyperus rotundus* leaves. *Int J Curr Biotechnol* 2: 19-23

