Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

GC-MS analysis of organic fractions of *Chrozophora tinctoria* (L.) A.Juss. and their prokinetic propensity in animal models

Análise por GC-MS de frações orgânicas de *Chrozophora tinctoria* (L.) A.Juss. e sua propensão procinética em modelos animais

A. A. Sher[a], A. Iqbal[a], M. Adil[b], S. Ullah[a]*, S. Bawazeer[c], M. K. Binmahri[d], L. Z. Zamil[e] and M. Irfan[f,g,h]

[a]Islamia College University, Deparment of Botany, Peshawar, Pakistan
[b]Qurtaba University Peshawar, Department of Botany, Peshawar, Pakistan
[c]Umm Al-Qura University, Faculty of Pharmacy, Department of Pharmacognosy, Makkah, Kingdom of Saudi Arabia
[d]Ministry of Health, Rabigh General Hospital, Emergency Department, Peshawar, Saudi Arabia
[e]Ministry of Health King abdulaziz hospital, Makkah, Kingdom of Saudi Arabia
[f]Abdul Wali Khan University, Department of Botany, Mardan, Pakistan
[g]University of Swabi, Department of Botany, Swabi, Pakistan
[h]Missouri Botanical Garden, St. Louis, Missouri, USA

Abstract

Chrozophora tinctoria (L.) A.Juss. is herbaceous, monecious annual plant used traditionally to cure gastrointestinal disorders. The present study was carried out to find the bioactive compounds by Gas Chromatography-Mass Spectrometry, the acetylcholinesterase inhibitory potential acute toxicity, and emetic activity present in the ethyl acetate fraction of *Chrozophora tinctoria* (*EAFCT*) and dichloromethane fraction of *Chrozophora tinctoria* (*DCMFCT*). The compounds detected in both fractions were mostly fatty acids, with about seven compounds in *EAFCT* and 10 in *DCMFCT*. These included pharmacologically active compounds such as imipramine, used to treat depression, or hexadecanoic acid methyl ester, an antioxidant, nematicide, pesticide, hypocholesterolemic, 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- is used as a cancer preventive, antiarthritic, antihistaminic, hepatoprotective, insectifuge, nematicide, Pentadecanoic acid, 14-methyl-, methyl ester have antifungal, antimicrobial and antioxidant activities, 10-Octadecanoic acid, methyl ester have the property to decrease blood cholesterol, Antioxidant and antimicrobial, 1-Eicosanol is used as an antibacterial, 1-Hexadecene has antibacterial, antioxidant, and antifungal activities. Both *DCMFCT* and *EAFCT* fractions inhibited acetylcholinesterase (AChE) activity with IC_{50} values of 10 µg and 130 µg, respectively. Both the fractions were found to be toxic in a dose-dependent manner, inducing emesis at 0.5g onward and lethargy and mortality from 3–5 g upwards. Both the fractions combined with distilled water showed highly emetic activity. The significant increase in the number of vomits was shown by *EAFCT* plus distilled water which are 7.50±1.29, 7.25±3.10, and 11.75±2.22 number of vomits at 1g, 2g, and 3g/kg concentration respectively, while *DCMFCT* plus distilled water showed 5.25±2.22, 7.50±2.52 and 10.25±2.22 number of vomits at 1g, 2, and 3g/kg correspondingly. The antiemetic standard drug metoclopramide has a higher impact against the emesis induced by both the fractions than dimenhydrinate. Metoclopramide decreases the number of vomits caused by *EAFCT* to 1.00±0.00, 2.00±0.00, 4.00±1.00 at 1g, 2, and 3g/kg sequentially, while dimenhydrinate decreases the number of vomits to 1.33±0.58, 2.33±1.15, 4.33±0.58 at 1g, 2, and 3g respectively. In the same way, Metochloprimide decreases the number of emesis caused by DcmCt from 5.25±2.22, 7.50±2.52, 10.25±2.22 to 1.33±0.58, 2.33±1.1, 4.33±0.58 at 1g, 2, and 3g/kg concentrations. The present study is the first documented report that scientifically validates the folkloric use of *Chrozophora tinctoria* as an emetic agent.

Keywords: *Chrozophora tinctoria,* GCMS, acetyl cholinesterase, acute toxicity, emetic activity.

Resumo

Chrozophora tinctoria (L.) A.Juss. é uma planta anual herbácea, monoica, usada tradicionalmente para curar distúrbios gastrointestinais. O presente estudo foi realizado para encontrar os compostos bioativos por Cromatografia Gasosa-Espectrometria de Massa (GC-MS), a toxicidade aguda do potencial inibitório da acetilcolinesterase e a atividade emética presente na fração acetato de etila de *Chrozophora tinctoria* (EAFCT) e fração diclorometano de *Chrozophora tinctoria* (DCMFCT). Os compostos detectados em ambas as frações foram principalmente ácidos graxos, com cerca de sete compostos em EAFCT e 10 em DCMFCT. Estes incluíam compostos farmacologicamente ativos, como a imipramina, usada para tratar a depressão, ou éster metílico do ácido hexadecanoico, um antioxidante, nematicida, pesticida, hipocolesterolêmico, ácido 9,12,15-octadecatrienoico, éster etílico, (Z,Z,Z)- é usado como

*saqibullahstd@icp.edu.pk
Received: January 31, 2022 – **Accepted:** April 04, 2022

preventivo do câncer, antiartrítico, anti-histamínico, hepatoprotetor, inseticida, nematicida, ácido pentadecanoico, 14-metil-, éster metílico tem atividades antifúngicas, antimicrobianas e antioxidantes, ácido 10-octadecanoico, éster metílico tem a propriedade de diminuir o colesterol no sangue, antioxidante e antimicrobiano, o 1-Eicosanol é usado como antibacteriano, o 1-Hexadeceno possui atividades antibacteriana, antioxidante e antifúngica. Ambas as frações DCMFCT e EAFCT inibiram a atividade da acetilcolinesterase (AChE) com valores de IC50 de 10µg e 130µg, respectivamente. Ambas as frações foram consideradas tóxicas de maneira dose-dependente, induzindo vômitos a partir de 0,5g e letargia e mortalidade de 3g a 5g para cima. Ambas as frações combinadas com água destilada apresentaram atividade altamente emética. O aumento significativo no número de vômitos foi demonstrado pelo EAFCT mais água destilada, que são 7,50±1,29, 7,25±3,10 e 11,75±2,22 número de vômitos nas concentrações de 1g, 2g e 3g/kg, respectivamente, enquanto DCMFCT mais água destilada mostrou 5,25±2,22, 7,50±2,52 e 10,25±2,22 número de vômitos em 1g, 2g e 3g/kg, respectivamente. A droga padrão antiemética metoclopramida tem um impacto maior contra a emese induzida por ambas as frações do que o dimenidrinato. A metoclopramida diminui o número de vômitos causados por EAFCT para 1,00±0,00, 2,00±0,00, 4,00±1,00 a 1g, 2g e 3g/kg, sequencialmente, enquanto o dimenidrinato diminui o número de vômitos para 1,33±0,58, 2,33±1,15, 4,33± 0,58 a 1g, 2g e 3g, respectivamente. Da mesma forma, a metocloprimida diminui o número de vômitos causados por DcmCt de 5,25±2,22, 7,50±2,52, 10,25±2,22 para 1,33±0,58, 2,33±1,1, 4,33±0,58 nas concentrações de 1g, 2g e 3g/kg. O presente estudo é o primeiro relato documentado que valida cientificamente o uso folclórico de *Chrozophora tinctoria* como agente emético.

Palavras-chave: *Chrozophora tinctoria*, GC-MS, acetilcolinesterase, toxicidade aguda, atividade emética.

1. Introduction

Emesis is an involuntary and irritant activity that results in the forceful ejection of stomach contents and food materials from the stomach through the mouth (Ahmed et al., 2013). The ejection of contents of the stomach and upper gastrointestinal tract through the mouth is called vomiting and the sensation to vomit is called nausea (Tanihata et al., 2000). Vomiting serves as a protective reflex is a complex process coordinated by the vomiting centre located in the medulla oblongata of the brain area postrema, a medullary structure in the brain is present on the floor of the 4th ventricle called chemoreceptor trigger zone has various chemosensors which controlled the vomiting center. The chemoreceptor trigger zone (CTZ) is mostly activated by certain neurotransmitters in the brain (serotonin, acetylcholine, histamine, norepinephrine, Met-enkephalin, Leu-enkephalin), toxins, nicotine, and dopamine agonists like apomorphine which leads to vomiting. Motion sickness, morning sickness (vomiting during pregnancy), overeating, bloated stomach, and inflamed abdominal organs activate vomiting without the intervention of CTZ (Silbernagl et al., 2009). The chemoreceptor trigger zone (CTZ) that gives a signal to the vomiting centre to start emesis includes the vestibular system, the higher centre in the thalamus and cortex, and the GI tract (MacDougall & Sharma, 2020). Other emetogenic are copper sulphate ($CuSO_4$, oral), fresh juice of *Brassica compestris* (oral), and cisplatin (I.V, I.M). Emesis also occurred during 1st trimester of pregnancy, postoperative procedures, indigestion of toxicants, Radio surgery, Cancer chemotherapy, and drug side effects (Hussain et al., 2015).

Medicinal plants are a rich source of bioactive compounds that are used for the synthesis of drugs. These active compounds obtained from leaf, root, shoot, flowers, skin, or whole plant have direct or indirect therapeutic effects (Jamshidi-Kia et al., 2018). These bioactive natural compounds are known as phytochemicals. These phytochemicals are the secondary plant metabolites that prevent the plant from natural risks like drought, stress, pollution, pathogenic attacks, and UV exposure. These compounds are easily available and have low cost and low toxicity. The most commonly occurring phytochemicals are alkaloids, sterols, flavonoids, phenols, terpenoids, tannins, saponins, and lignans. These biologically active compounds are not required by human beings to sustain their life but are important to prevent some diseases. (Nyamai et al., 2016). Antiemetic agents extracted from such plants act against emesis. Antiemetic therapy aims to treat or manage nausea and vomiting associated with various ailments. This task is flourished for patients who undergo chemotherapy or radiation therapy and is based on clinical and basic research that has helped to overcome emesis for the last 20 years (Gralla et al., 1999).

Chrozophora tinctoria is commonly known as 'dyer's-croton or 'turnsole belongs to Euphorbiaceae family. It is an annual plant mostly found in Africa, Asia and Europe; in Pakistan it is dominated in tropical and temperate regions. The height of the plant varies from 40 to 60 cm and bears flowers from June to September. It produces the "turnsole" used as a colorant in foodstuff and is famous for producing dye substances and flavonoids. It has been also evaluated that besides flavonoids, the plant also contains alkaloids, xanthones, coumarins, glycosides, and diterpenoids. The different fractions of *Chrozophora tinctoria* also showed antibacterial and antifungal activity, the methanolic fractioned sample has antipyretic and anti-nociceptive activity, as it reduces abdominal pain and temperature (Sher et al., 2018). Various flavonoids isolated from fractions showed the antiosteoporosis influence; flavonoids (rutin) improves the proliferation and ossification (Abdel-Naim et al., 2018). Snafi (2015) documented that *Chrozophora tinctoria* is rich in secondary metabolites, therefore it showed antibacterial, antioxidant, antifungal and cytotoxic activities. The fruits extract is used as eye drops, roots and leaves were used as antidiabetic and to treat hair loss. It was also detected that hypoglycaemic response in rats was caused by the aerial parts of *Chrozophora tinctoria*. Different species of *Chrozophora* were ethnomedicinally and traditionally used to cure mouth ulcer, skin burns, fever, abdominal pain, jaundice, joints pain, menstrual problems, expel worms and migraine. Species like *Chrozophora tinctoria* is used as an emetic, antipyretic, cathartic and to treat warts. In

Sudan *Chrozophora rottleri* is traditionally used to improve the healing of wounds, while in Saudi Arabia and India it is used for purifying blood. In Ethiopia and Senegal their seeds and leaves were used as a laxative. In Nepal fruit juice is used for the treatment of cough and cold (Khare, 2007). *Chrozophora senegalensis* is used as an astringent, in Senegal the decoction of *Chrozophora senegalensis* roots are used to treat diarrhea in suckling babies (Etkin, 1997). *Chrozophora plicata* is used as an emetic and cathartic.

In current study the ethyl acetate fraction of *Chrozophora tinctoria* (*EAFCT*) and dichloromethane fraction of *Chrozophora tinctoria* (*DCMFCT*) were subjected to confirm their emetic potential.

2. Materials and methods

2.1. Plant collection and identification

Full mature plants of *Chrozophora tinctoria* were collected from their natural habitats in Mohmand Agency (34.225^0 N to 71.482^0 E), Khyber Pakhtunkhwa, Pakistan. After their collection, the mature plant was identified in the flora of Pakistan (Taxon I.D 220002849). This plant was also authenticated in the Department of Botany, Islamia College, University, Peshawar, Pakistan with a voucher specimen (Icp-2021/49) for *Chrozophora tinctoria* has been deposited in (ICP). The collected plant was washed with tap water to remove dust and dirt and was spread on blotting papers to get dried at room temperature. After drying the plant was cut into fine pieces and was powdered in an electric grinder.

2.2. Extraction and fractionation

10kg powdered plant was macerated in methanol with random shaking for three weeks. It was then filtered using Whatman's filter paper No 1. The filtrate was concentrated by using a rotary evaporator to obtain crude extract. Further, the dried methanolic extract was fractioned with different solvents, following the Solvent-Solvent fractionation method, to get our desired fractions of dichloromethane and ethyl acetate. Their order of fractionation was from *n*-hexane to dichloromethane, ethyl acetate, and *n*-Butanol respectively. The dichloromethane fraction of *Chrozophora tinctoria* (*DCMFCT*) and ethyl acetate fraction of *Chrozophora tinctoria* (*EAFCT*) were used for further activities (Bakht et al., 2011).

2.3. Chemicals and solvents

The chemicals/drugs used in the present study were, Gum acacia (Shreeji Pharma International), Castor oil (Karachi Pharmaceuticals Laboratory, Karachi, Pakistan), Immodium® (ASPIN Pharma PVT. LTD, Karachi, Pakistan), distilled water, normal saline (Shahzeb Pharmaceutical, Haripur, KPK, Pakistan), methanol, *n*-Hexane, dichloromethane, ethyl acetate and *n*-Butanol (Master chemical supplier, Karachi, Pakistan), The chemicals/drugs used in the present study were Maxolon® (Valeant Pharmaceutical international, inc, Pakistan), Gravinate® (The Searle company (PVT) LTD, Pakistan),

2.4. Instruments

Chopper machine, electric grinder, Large and small flasks, Separating funnel, A rotary evaporator (Model RE-111, Bochi Switzerland), analytical balance (Shimadzo analytical balance), glass funnel, filter papers, feeding tube, water bath (Thermostatic controlled-STD/GMP), magnetic stirrer (H3760-S Digital magnetic stirrer), Large and small cages, Petri dishes.

2.5. Animals

Healthy and mature pigeons of both sexes were taken weighing 240-340g and were given locally available standard food; Millet + Wheat grains, freshwater and a light/dark cycle for 12/12 hours for 5-7 days. On the day of the experiment, the pigeons were weighed and examined again for good health. Pigeons observed as unhealthy and immature were removed from the experiment. After selection animals were placed in separate cages to get the individual data of each pigeon (Muhammad et al., 2020).

2.6. Ethical approval

The study was approved by the ethical board of the Islamia College, University, Peshawar, Pakistan. The ethical approval no. was EC/Bot/ICP-673.

2.7. Gas Chromatography-Mass Spectrometry (GC-MS)

To identify the different phytochemicals present in the ethyl acetate fraction of *Chrozophora tinctoria* (*EAFCT*) and dichloromethane fraction of *Chrozophora tinctoria* (*DCMFCT*), the sample was subjected to GC-MS. The sample was checked using Thermo Scientific (DSQ-II) GC. The gas chromatography (GC) was furnished with a 30 meter lengthy TR-5MS capillary column, 0.25µm thick film, and 0.25mm of internal diameter. Helium (He) was used as carrier gas with 1ml/min of flow rate. The injection device was run in a split mode having 250^{0}C. 1µl sample of the volume was administered with a beginning oven temperature of 50°C and maintained for 2 minutes then the temperature was raised to 150 °C with a flow rate of 08°C/min. After this with an interval of 15°C/min temperature was elevated to 300°C and controlled for 5 minutes (Alam et al., 2014).

2.8. Pharmacological activities

2.8.1. In-vitro experiments

2.8.1.1. Acetylcholinesterase assay

The ethyl acetate fraction of *Chrozophora tinctoria* (*EAFCT*) and dichloromethane fraction of *Chrozophora tinctoria* (*DCMFCT*) were tested to inhibit acetylcholinesterase (AChE) using a spectrophotometer based on Ellman's method (Ellman et al., 1961). In this activity, the acetylthiocholine iodide is hydrolyzed by the respective enzymes producing thiocholine which reacts with DTNB (Ellman's reagent) to produce 5-thio-2 nitrobenzoate and 2-nitrobenzoate-5-mercaptothiocholine detected by spectrophotometer (412 nm). The concentrations of ethyl acetate fraction of

the *Chrozophora tinctoria* (*EAFCT*) prepared were 1000, 500, 250, and 125 µg/ml. The concentrations of positive control known as Galantamine were also prepared in the same manner, the mixtures were incubated at 37°C for 20 minutes. The enzyme inhibited by samples and positive control were calculated from the absorption rate with change in time (Ayaz et al., 2014).

The percent inhibition was calculated as Equation 1 and Equation 2:

$$\text{Enzyme inhibition (\%)} = 100 - \text{percent enzyme activity} \quad (1)$$

$$\text{Enzyme activity (\%)} = 100 \times V / V\text{max} \quad (2)$$

Where (Vmax) is an enzyme activity in the absence of an inhibitor

Data were collected in triplicates, the IC_{50} value was calculated by using the excel program (linear regression analysis between the percentage of inhibition and fraction concentrations)

2.8.2. In-vivo experiments

2.8.2.1. Acute toxicity

Acute toxicity of *EAFCT* and *DCMFCT* was performed according to the protocol of (Joshi et al., 2007) with slight modification. The pigeons were divided into two groups (n=8). One group received a fraction while the other group serving as positive control received distal water (6ml/kg, P.O). The fractions was administered orally with 0.3, 0.5, 1, 2, 3, 4, and 5g/kg as a single dose using a feeding tube to different groups. All the animals were observed for toxic symptoms i.e. diarrhea, emesis, lethargy, and mortality for about 72 hours.

2.8.2.2. Emetic activity

To find out the emetic activity of the ethyl acetate fraction of *Chrozophora tinctoria* (*EAFCT*) and dichloromethane fraction of *Chrozophora tinctoria* (*DCMFCT*) the pigeons were divided into different groups (n=8). Group 1st was declared as negative control and groups 2nd, 3rd and 4th received individual plant samples with a concentration of 1g, 2g, and 3g. After 30 minutes dimenhydrinate (2mg/kg, IM) was injected. First jerk time, first vomit time, the number of jerks, the number of vomits, and the weight of vomit were noted. The same activity was repeated for metoclopramide (2mg/kg, I.M) replacing dimenhydrinate.

3. Results

3.1. GC-MS

3.1.1. EAFCT

The *EAFCT* was checked for phytochemicals, the *EAFCT* displayed several peaks, which exhibit different bioactive compounds present in the sample (Figure 1). These peaks were matched with the database of the spectrum of known components present in the GC-MS library.

GC-MS analysis of *EAFCT* showed the presence of many bioactive compounds at different retention times (min). about 9 compounds were identified. These compound are Hexadecanoic acid, ethyl ester (0.07%), Hexadecanoic acid, methyl ester (0.06%), 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (0.03%), Pentadecanoic acid, 14-methyl-, methyl ester (0.02%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (0.01%), Imipramine (0.01%), D-Tyrosine, 3-hydroxy- (0.01%), 1-Tricosanol (0.01%) and Nonanoic acid, 9-(0-propylphenyl)- methyl ester (0.01%) Table 1.

3.1.2. DCMFCT

The *DCMFCT* displayed several peaks, which exhibit different bioactive compounds present in the fraction as shown in Figure 2. These peaks were matched with the database of the spectrum of known components present in the GC-MS library.

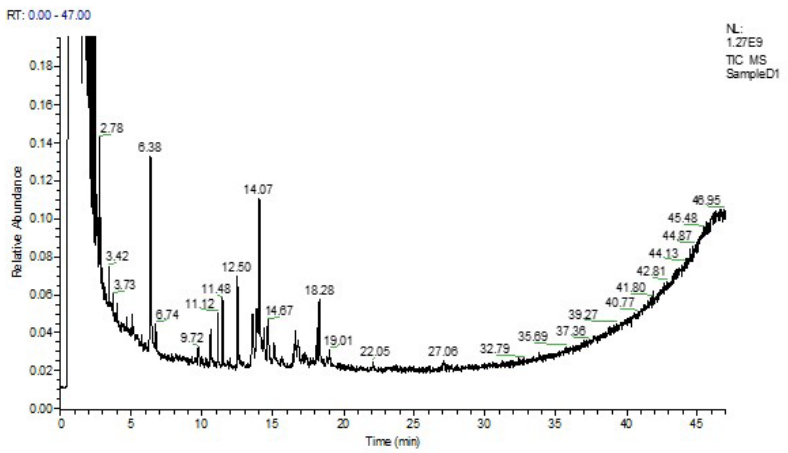

Figure 1. GC-MS chromatogram of the ethyl acetate fraction of *Chrozophora tinctoria* (*EAFCT*) the numbers showed the retention times of various compounds.

Table 1. Bioactive compound identified in *EAFCT* through GC-MS.

RT (min)	SI	RSI	Area %	Prob.	Compound name	Formula	MW	Library
2.78	783	980	0.01	93.57	Imipramine	$C_{19}H_{24}N_2$	280	nist_msms
3.42	499	591	0.01	16.41	D-Tyrosine, 3-hydroxy-	$C_9H_{11}NO_4$	197	MAINLIB
6.38	905	914	0.06	76.55	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	replib
9.72	521	569	0.01	3.22	1-Tricosanol	$C_{23}H_{48}O$	340	replib
11.12	684	870	0.01	22.81	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	MAINLIB
12.5	865	889	0.02	48.69	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	MAINLIB
14.07	846	866	0.07	80.32	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	MAINLIB
14.67	612	652	0.01	45.49	Nonanoic acid, 9-(0-propylphenyl)- methyl ester	$C_{19}H_{30}O_2$	290	MAINLIB
18.28	728	749	0.03	12.14	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	replib

Keys: RT (Retention Time), SI (Similarity Index), RSI (Relative Similarity Index), Prob (Probability), MW (Molecular weight).

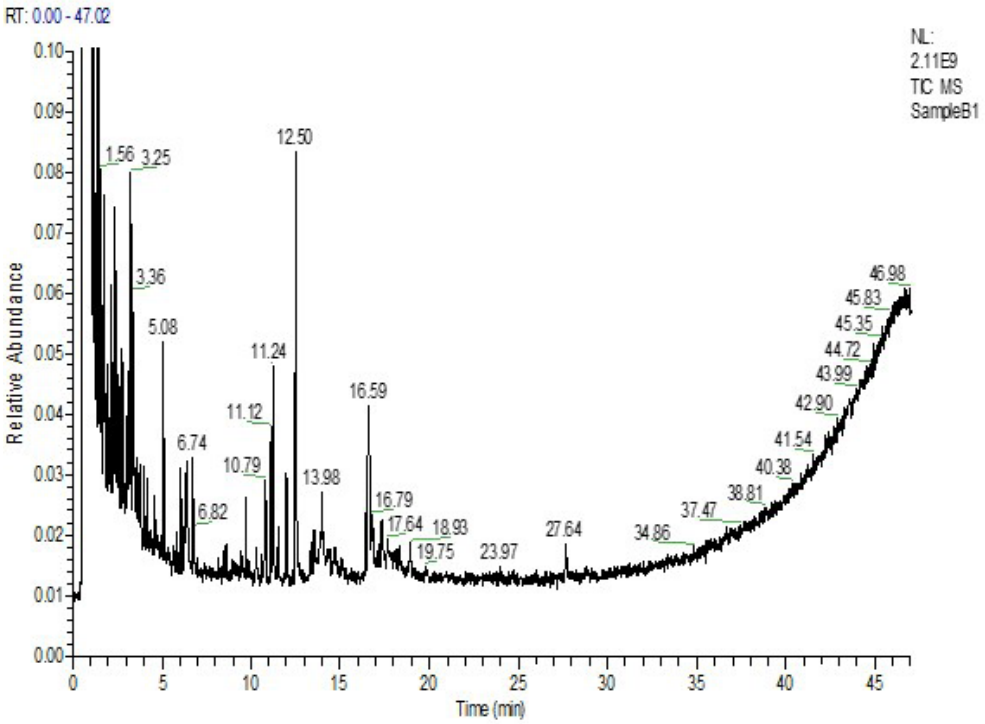

Figure 2. GC-MS chromatogram of the dichloromethane fraction of *Chrozophora tinctoria* (*DCMFCT*) the numbers show the retention times of various compounds.

GC-MS analysis of *DCMFCT* showed the presence of many bioactive compounds at different retention times (min) as shown in Table 2 that are Hexadecanoic acid, methyl ester (0.06%), Hydroperoxide, 1-ethyl butyl (0.06%), 10-Octadecanoic acid, methyl ester (0.05%), Silane, chlorodiisopropylmethyl- (0.04%), 1-Eicosanol (0.03%), 1-Hexadecene (0.01%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (0.01%), 9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)- (0.01%), 17-Pentatriacontene (0.01%) and 1,2-Benzenedicarboxylic acid, diisooctyl ester (0.01%).

3.2. Acetylcholinesterase Inhibitory activity

3.2.1. EAFCT

In Table 3 the different concentrations i.e. 1000, 500, 250, and 125µg/ml of *EAFCT* were tested for their Inhibitory potential of acetylcholinesterase (AChE). 1000 µg/ml showed the highest percent AChE inhibition of 93.33±1.53 followed by 500 µg/ml, 250 µg/ml, and 125 µg/ml. almost similar results were shown by Standard Galantamine. the inhibitory concentration (IC_{50}) of ethyl acetate fraction showed the strongest AChE inhibition activity with

Table 2. Bioactive compounds identified in *DCMFCT* through GC-MS.

RT (min)	SI	RSI	Area %	Prob	Compound name	Formula	MW	Library
1.56	742	812	0.06	51.92	Hydroperoxide, 1-ethyl butyl	$C_6H_{14}O_2$	118	MAINLIB
3.25	657	722	0.04	40.54	Silane, chlorodiisopropylmethyl-	$C_7H_{17}ClSi$	164	MAINLIB
5.08	712	891	0.01	4.42	1-Hexadecene	$C_{16}H_{32}$	224	MAINLIB
10.79	686	757	0.01	26.35	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	MAINLIB
12.5	912	919	0.06	72.93	Hexadecanoic acid, methyl ester	$C_{17}H_{34}0_2$	270	MAINLIB
13.98	728	893	0.03	6.03	1-Eicosanol	$C_{20}H_{42}O$	298	replib
16.59	775	791	0.05	8.39	10-Octadecanoic acid, methyl ester	$C_{19}H_{36}O_2$	296	MAINLIB
17.64	674	714	0.01	25.03	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl) oxy]propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$	496	replib
18.93	651	675	0.01	6.21	17-Pentatriacontene	$C_{35}H_{70}$	490	MAINLIB
27.64	727	899	0.01	41.12	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	replib

Keys: RT (Retention Time), SI (Similarity Index), RSI (Relative Similarity Index), Prob (Probability), MW (Molecular weight).

Table 3. Percent inhibition of acetylcholinesterase by *EAFCT* and *DCMFCT*.

Compound name/ Plant name	Extracts/Fractions	Concentration (µg/ml)	% AChE inhibition	IC_{50}
Galantamine	Standard	1000	95.67±2.52	5
		500	87.33±2.52	
		250	82.67±3.06	
		125	77.00±3.00	
Chrozophora tinctoria	Ethyl acetate (EAFCT)	1000	93.33±1.53	10
		500	87.33±3.06	
		250	80.67±2.08	
		125	73.00±4.58	
	Dichloromethane (DCMFCT)	1000	68.33±2.52	130
		500	63.00±3.61	
		250	57.67±2.31	
		125	48.67±2.08	

Keys: Values are expressed as mean ± SEM. Statistical significance was determined using IC_{50} values through Biostata software. IC_{50}= Half-maximal Inhibitory concentration.

IC_{50}=10 µg/ml as compared to standard Galantamine which showed IC_{50}=05 µg/ml. *EAFCT* exhibited the strongest AChE inhibition activity at all concentrations.

3.2.2. DCMFCT

The *DCMFCT* was analyzed for AChE Inhibitory activity in the same manner and it was evaluated that the fraction showed moderate inhibition activity (68.33±2.52%) at a concentration of 1000 µg/ml as compared with Galantamine (95.67±2.52%). The inhibitory concentration (IC_{50}) value recorded for *DCMFCT* reveals satisfactory AChE inhibitory concentration (130µg/ml) as compared with Galantamine in Table 3. the fraction showed the dose-dependent inhibition. The percentage of acetylcholinesterase inhibition increases with an increase in concentration.

3.3. Acute toxicity.

3.3.1. EAFCT

The acute toxicity of *EAFCT* was determined in pigeons at doses of 0.3g, 0.5g, 1g, 2g, 3g, 4g and 5g/kg (P.O), the results presented in Table 4 illustrated that at a concentration of 0.3g/kg no emesis, diarrhea, lethargy and mortality was found. But at a concentration of 0.5g/kg and onward it was noticed that the toxic effect of the sample appeared in case of vomiting only, where the total number of vomiting was 2.00±0.82. Further it was noted that the toxic effect of the plant as diarrheal was recorded at a concentration of 1g/kg with 8.33±4.51 number of wet stool and concentrations of 4g/kg and 5g/kg all the groups were found to emetic, diarrhoeal with more lethargic and 25.00% mortal. The negative control i.e. distal water (D.W) group showed

Table 4. Acute toxicity of ethyl acetate and dichloromethane fraction of *Chrozophora tinctoria* in Pigeons.

Sample		Dose (g).(ml)/kg Total No. of vomits	Emesis	Diarrhea	Lethargy	Mortality (%)
			Total No. of wet stools			
Distilled Water		6	0.00±0.00	0.00±0.00	-	-
Chrozophora tinctoria	*EAFCT*	0.3	0.00±0.00	0.00±0.00	-	-
		0.5	2.00±0.82	0.00±0.00	-	-
		1	6.00±2.65*	8.33±4.51	-	-
		2	6.67±2.52*	10.00±3.00*	-	-
		3	8.00±2.00***	10.67±3.06**	Less	-
		4	9.33±1.53***	12.33±2.52***	More	25
		5	11.00±3.00***	14.00±4.00***	More	25
	DCMFCT	0.3	0.00±0.00	0.00±0.00	-	-
		0.5	2.33±0.58	0.00±0.00	-	-
		1	6.33±1.53*	6.00±3.00	-	-
		2	7.00±1.73**	8.67±2.08	-	-
		3	9.67±2.08***	9.33±2.52*	Less	-
		4	10.00±2.65***	9.67±4.04*	More	25
		5	11.33±2.31***	11.00±1.00**	Most	25

Keys: Values are expressed as mean ± SEM. Statistical significance was determined with one-way ANOVA followed by Tukey's multiple comparison test (using Graph Pad Prism 6.01 software); $^{*}p \leq 0.05$ was considered statistically significant. (** $p \leq 0.01$, *** $p \leq 0.001$).

no emesis, diarrhea, lethargy, and mortality. It has been observed that the ethyl acetate fraction is less toxic at 0.5g/kg and is more toxic after 3g/kg.

3.3.2. DCMFCT

In the same way, the acute toxicity of *DCMFCT* was recorded and it was investigated that the *DCMFCT* showed its at 0.5g/kg concentration where the only emesis was recorded and the total number of vomiting was 2.33±0.58, similarly from 1g and onward both the emesis and diarrhea was noticed as shown in Table 4. The lethargy was less at 3g, more at 4g, and most at 5g dose. The percent mortality was recorded at 4g and 5g that was 25.00% and 25.00% respectively.

3.4. Emetic activity

3.4.1. Dimenhydrinate emesis Inhibition

3.4.1.1. EAFCT

The ethyl acetate fraction of *Chrozophora tinctoria* + distal water (*EAFCT*+D.W) accelerated the emetic episodes at all of the tested doses as shown in Table 5. 1st jerk time and 1st vomit time were decreased and the number of jerks, the number of vomits, and weight of vomits were increased by (*EAFCT* +D.W) in a dose-dependent manner. The *EAFCT* was also given to the groups who were administered dimenhydrinate (2mg/kg I.M) 30 minutes before. It was revealed that the emesis was not stopped by the administration of dimenhydrinate (Dim) but only decrease the emesis and its responses. All of the tested doses demonstrated a significant ($P< 0.01$) increase in 1st jerk time as well as a significantly ($P<0.1$) 1st vomiting time duration. The number of jerks and vomits were also significantly ($P< 0.01$) increased.

3.4.1.2. DCMFCT

The emetic potential of *DCMFCT* was tested in pigeons as shown in Table 6. It was checked out that the fraction with distal water showed a prominent emetic activity at all three (1g, 2g, and 3g) doses by reducing the 1st jerk time and 1st vomit time and increasing the number of jerks, vomits and weight of vomits, the dichloromethane fraction of *Chrozophora tinctoria* + distal water (*DCMFCT* + D.W) was highly potent at 3g/kg (b.w) dose, showing its dose-dependent property as shown in the table, similarly, the fraction was also given (P.O) to the pigeons who were injected dimenhydrinate (2mg/kg I.M) 30 minutes before. It was noted that the dichloromethane fraction of *Chrozophora tinctoria* + dimenhydrinate (*DCMFCT* + Dim) showed minimum emetic activity as compared to (*DCMFCT* + D.W), dimenhydrinate decrease the emetic effect of the plant to some extent as compared to fraction and distal water. but did not stop or sufficiently decrease the emetic effect of the plant at all doses, because the antiemetic agent (dimenhydrinate) only delayed the 1st jerk time and 1st vomit time and decrease the number of jerks and vomit but did not stop it completely.

Table 5. Emetic activity of *EAFCT* against Dimenhydrinate.

Sample	Dose (g).(ml) /kg	1st Jerk time (min)	1st vomit time (min)	Number of jerks	Number of vomits	Weight of vomits
D.W + *EAFCT*	1	16.25±2.36	19.25±1.71	3.75±1.50	7.00±0.82	5.75±2.08
	2	13.25±2.50	17.00±2.58	4.00±1.83	8.25±1.71	6.88±2.56
	3	11.50±2.65	15.25±3.86	4.50±2.38	10.50±2.65	8.38±1.89
Administration of exact (P.O) after 30 minutes of dimenhydrinate (2mg/kg I.M).						
D.W + Dim	6	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
EAFCT+ Dim	1	20.33±1.53***	25.00±1.00***	1.67±0.58	5.00±2.00**	3.63±1.52**
	2	21.00±1.00***	24.33±1.53***	2.33±0.58**	5.00±2.00**	3.07±1.01*
	3	19.33±1.53***	21.33±2.31***	3.33±1.15***	6.00±2.00**	4.33±1.75***

Keys: Data were presented as mean ± SEM, one-way ANOVA followed by Dunnett's test was applied. * [p< 0.05]; ** [p<0.01) and *** [p<0.001) indicating the level of significance. D.W= Distilled water. Dim= dimenhydrinate. P.O= per Orally, I.M= Intramuscularly.

Table 6. Emetic activity of DCMFCT against Dimenhydrinate.

Sample	Dose (g).(ml) /kg	1st Jerk time (min)	1st vomit time (min)	Number of jerks	Number of vomits	Weight of vomits
DCMFCT +D.W	1	7.75±2.50	10.25±3.30	4.25±1.71	7.25±2.22	4.95±1.90
	2	5.75±1.71	7.75±2.22	5.75±2.63	8.75±1.50	6.85±1.62
	3	3.50±1.29	5.75±1.71	7.50±1.91	9.50±1.73	8.05±1.46
Administration of exact (P.O) after 30 minutes of Dimenhydrinate (2mg/kg I.M).						
Dim+D.W	6	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
DCMFCT + Dim	1	18.00±1.00***	21.00±1.00***	2.33±0.58**	5.00±2.00**	3.73±1.62**
	2	17.00±2.00***	20.00±2.00***	3.00±1.00***	5.33±1.53**	2.63±0.40*
	3	14.00±1.00***	16.67±2.08***	4.00±1.00***	7.00±3.00***	4.50±1.57***

Keys: Data were presented as mean ± SEM, one-way ANOVA followed by Dunnett's test was applied. * [p< 0.05]; ** [p<0.01) and *** [p<0.001) indicating the level of significance. Dim= dimenhydrinate. D.W= distilled water, P.O= per orally. I.M= Intramuscularly.

3.4.2. Metoclopramide emesis inhibition

3.4.2.1. EAFCT

EAFCT + distal water was given (p.o) to 1st three groups of animals (n=8) at doses of 1g, 2g, and 3g, and the behaviors like 1st Jerk time, 1st vomit time, number of jerks, number of vomits were observed along with the weight of vomits, it was noted that *EAFCT* + D.W significantly decrease the 1st jerk time and 1st vomit time and boosted the number of jerks and number of vomits from 1g to 3g. Metoclopramide (MTC) (2mg/kg) was administered intramuscularly to all the remaining groups including negative control (D.W). After 30 minutes *EAFCT* was given to different groups at a concentration of 1g, 2g, and 3g as shown in Table 7. it was perceived that emesis and response (jerk) to emesis started from 1g onward. the 1st jerk and vomit time decreased from low dose to high dose (from 1g to 3g), decrease in 1st jerk and vomit time signals induction of emesis. Similarly, the number of jerks and vomits increases with an increase in dose concentration. But the number of vomits and weight vomited material was not more. And the emesis stopped after some time, this might be due to the inhibition potential of metoclopramide (2mg/kg), which reduces or stopped the emesis.

3.4.2.2. DCMFCT

The *DCMFCT* + distilled water showed significant emetic potential at 1g, and the most significant emetic activity at 3g/kg (Table 8). Further these doses were tested against the antiemetic drug metoclopramide. metoclopramide (2mg/kg I.M) was injected into the remaining groups including distal water. It was investigated that the antiemetic drug metoclopramide reduces the emetic effect of the *DCMFCT*. *DCMFCT* + Mtc increased the 1st jerk time and 1st vomit time and decreases the number of jerks and vomits as compared to *DCMFCT* + distilled water and distilled water group.

4. Discussion

Chrozophora tinctoria belongs to family Euphorbiaceae is a highly medicinal plant that is traditionally used to cure certain diseases viz. fever, mouth ulcer, skin disorders, jaundice, joint pain, menstrual problems and wounds (Delazar et al., 2006). Moreover the plant is also used as an emetic and cathartic. Medicinal plants are the main source of bioactive compounds that are known as phytochemicals. Phytochemicals obtained from medicinal plants fight

Table 7. Emetic activity of *EAFCT* against metoclopramide.

Sample	Dose (g).(ml) /kg	1st Jerk time (min)	1st vomit time (min)	Number of jerks	Number of vomits	Weight of vomits
EAFCT + D.W	1	18.25±2.22	19.75±1.50	4.25±1.71	7.50±1.29	5.90±1.33
	2	14.25±3.10	15.50±2.65	5.50±2.08	7.25±3.10	5.73±1.42
	3	9.50±2.08	10.25±2.75	5.75±1.71	11.75±2.22	7.85±2.26
Administration of exact (P.O) after 30 minutes of metoclopramide (2mg/kg I.M).						
D.W	6	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
EAFCT+ Mtc	1	41.67±1.53***	43.67±1.53***	1.00±0.00	1.00±0.00*	0.50±0.20
	2	37.33±2.08***	42.00±2.00***	1.67±1.53*	2.00±0.00**	0.70±0.40*
	3	25.67±4.04***	30.33±3.06***	4.00±1.00***	4.00±1.00***	2.20±0.62***

Keys: Data were presented as mean ± SEM, and one-way ANOVA followed by Dunnett's test was applied. * [$p< 0.05$]; ** [$p<0.01$) and *** [$p<0.001$) indicating the level of significance. P.O= per orally, I.M= Intramuscular, D.W= Distilled water.

Table 8. The emetic activity of *DCMFCT* against metoclopramide.

Sample	Dose (g).(ml) /kg	1st Jerk time (min)	1st vomit time (min)	Number of jerks	Number of vomits	Weight of vomits
DCMFCT + D.W	1	8.25±2.87	9.75±3.30	3.25±1.26	5.25±2.22	3.93±1.42
	2	4.75±2.50	6.75±3.59	5.25±2.06	7.50±2.52	6.53±1.85
	3	2.75±2.22	3.75±1.71	6.75±1.71	10.25±2.22	7.73±1.67
Administration of exact (P.O) after 30 minutes of metoclopramide (2mg/kg I.M)						
Distal water	6	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
DCMFCT + Mtc	1	39.00±2.00***	41.33±2.08***	1.00±1.00	1.33±0.58	0.50±0.35
	2	37.00±2.00***	39.33±2.08***	2.33±1.15**	2.33±1.15***	1.10±0.10*
	3	24.67±4.51***	27.33±4.51***	3.33±0.58***	4.33±0.58***	2.43±0.95***

Keys: Data were presented as mean ± SEM, one-way ANOVA followed by Dunnett's test was applied. * [$p< 0.05$]; ** [$p<0.01$) and *** [$p<0.001$) indicating the level of significance. P.O= per Orally, I.M= Intramuscular.

against different diseases (Khan et al., 2019). About 9 compounds were identified in the ethyl acetate fraction of *Chrozophora tinctoria* (*EAFCT*) and similarly about 10 different compounds were detected in the dichloromethane fraction of *Chrozophora tinctoria* (*DCMFCT*) through GC-MS. Out of these total (9 and 10) compounds found in both the fractions, some compounds were found to have significant pharmacological activities. like Hexadecanoic acid, ethyl ester has hemolytic, nematicide, hypocholesterolemic, anti-androgenic, and antioxidant activities (Tyagi & Agarwal, 2017). Hexadecanoic acid, methyl ester have antioxidant, nematicide, pesticide, hypocholesterolemic, nematicide, hemolytic,5-Alpha reductase inhibitor properties (Sudha et al., 2013), 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- is used as a cancer preventive, antiarthritic, antihistaminic hepatoprotective, insectifuge, nematicide, anti-inflammatory, hypocholesterolemic, 5-Alpha reductase inhibitor, antiandrogenic, antihistaminic and anticoronary, Pentadecanoic acid, 14-methyl-, methyl ester has antifungal, antimicrobial and antioxidant activities (Elaiyaraja & Chandramohan, 2018). 3,7,11,15-Tetramethyl-2-hexadecane-1-ol have anti-inflammatory, cancer preventive, and antimicrobial property (Sudha et al., 2013), 1-Tricosanol have antibacterial and antifungal property (Tayade et al., 2013), Imipramine has the anti-depression property (Ramirez & Sheridan, 2016), 10-Octadecanoic acid, methyl ester have the property to decrease blood cholesterol, Antioxidant and antimicrobial. (Belakhdar et al., 2015), 1-Eicosanol is used as an antibacterial (Chatterjee et al., 2018), 1-Hexadecene has antibacterial, antioxidant, and antifungal activities (Belakhdar et al., 2015). Previously Delazar et al. (2006) documented five flavonoid glycosides including a new acylated flavones glycoside (*Chrozophorine*), similarly from the methanolic extract of the aerial parts of *Chrozophora tinctoria* by HPLC analysis. (Marzouk et al., 2016) reported 9 flavonoids, 2 phenolics and 1 steroid from the ethanolic extract of the aerial parts of *Chrozophora tinctoria*. The emetic potential of the fractions might be due to the presence of compounds like, 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- and 3,7,11,15-Tetramethyl-2-hexadecane-1-ol which are anti-cancer (Sudha et al., 2013). As most chemotherapeutics like cisplatin used as anticancer causes vomiting (Muhammad et al., 2020).

Acetylcholinesterase is the key enzyme in the blood and nervous system. The principal role of this enzyme is the stopping of nerve impulse transmission at cholinergic

synapses by the breaking down of neurotransmitter acetylcholine into choline and acetic acid. The inhibition of acetylcholinesterase is a promising strategy against Parkinson's diseases, myasthenia gravis, ataxia, senile dementia, and most common Alzheimer's disease (Mukherjee et al., 2007). Phytochemicals derived from plants have shown inhibitory action against acetylcholinesterase (Mathew & Subramanian, 2014). The chemoreceptor trigger zone (CTZ) is mostly activated by certain neurotransmitters in the brain (serotonin, dopamine, acetylcholine, histamine, norepinephrine, Met-enkephalin, Leu-enkephalin, GABA, and substance P) which leads to vomiting (Santana, 2016). acetylcholine is the most responsible neurotransmitter that stimulates the vomiting reflex through the afferent pathway. When the enzyme acetylcholinesterase is inhibited, the amount of acetylcholine (neurotransmitters) increases, this acetylcholine activates the chemoreceptor trigger zone which results in vomiting. The ethyl acetate fraction of *Chrozophora tinctoria* (*EAFCT*) significantly inhibits (IC_{50}=10µg/ml) the acetylcholinesterase (AChE). The fraction showed a dose-dependent inhibitory response. Percent AChE inhibitory activity of the fraction was comparable to standard galantamine (IC_{50}=05µg/ml) at all concentrations, whereas IC_{50} of the samples was slightly different. Similarly, the dichloromethane fraction of *Chrozophora tinctoria* (*DCMFCT*) showed anti-acetylcholinesterase activity (IC_{50}=130µg/ml) which was comparably less significant than *EAFCT* (IC_{50}=10µg/ml) and galantamine (IC_{50}=05µg/ml). The results confirmed that both the fractions inhibited acetylcholinesterase enzymes, which resulted in emesis.

Whenever an observer injects intramuscularly/ intravenously or gives orally a chemical substance or extract to an organism, different types of chemical reactions and a series of dose-related responses occur in the body of an organism. These responses are useful and are of our desire but in some cases, these responses are not advantageous and are harmful to the organisms, the types of toxicity tests performed for the investigation of new drugs by pharmaceutical researchers are chronic, acute, and sub-acute toxicity tests (Akhila et al., 2007). The acute toxicity test revealed that oral administration of a single 0.3g/kg dose of *EAFCT* and *DCMFCT* to pigeons did not show any toxicity or mortality. The toxicity in both fractions began from 0.5g/kg in the shape of vomiting and the more adverse effect of both the samples was found at 2g, 3g, 4g, and 5g/kg the mortality occurs at 4g/kg and 5g/kg.

Scientists are always trying to investigate natural products which are potent emetogenic with less or no side effects. Emesis is a reflex action that throws out the food contents/ toxic substances from the upper gastrointestinal tract. Similarly, the sensation to vomit is called Nausea, the nausea is pleasant nor painful (Andrews, 1992). *Chrozophora tinctoria* is also used as an emetic and cathartic. The *EAFCT* induced emesis in pigeons at a concentration of 1g, 2g and 3g the highest number of jerks. Vomits and weight of vomit was noted at 3g/kg the emetic activity of the plant was dose-dependent, the higher the concentration the higher was emetic activity, the emetic activity of the plant was decreased by inhibitor dimenhydrinate (2mg I.M) but did not stop emesis completely as shown in Table 5. In the same way, the emetic potential of the fraction was checked in pigeons with the antiemetic drug metoclopramide (2mg I.M). It was found that metoclopramide decreased the emetic potential of the plant more than dimenhydrinate (2mg I.M). It means that metoclopramide has a higher impact on the emesis induced by the plant than dimenhydrinate. Similar observations were noticed for *DCMFCT*. The *DCMFCT* was found to be more significantly emetic at all three doses (1g, 2g, and 3g/kg). Dimenhydrinate (2mg I.M) and metoclopramide (2mg I.M) were checked against the emesis induced by *DCMFCT* at all three doses (1g, 2g, and 3g/kg). it was observed that the antiemetic drug metoclopramide decreases the emetic potential of the *DCMFCT* more significantly than dimenhydrinate.

5. Conclusion

It was concluded that about 9 compounds were identified in the ethyl acetate fraction of *Chrozophora tinctoria* (*EAFCT*) and 10 different compounds were detected in the dichloromethane fraction of *Chrozophora tinctoria* (*DCMFCT*) having significant therapeutic qualities. Both the fractions inhibited Acetylcholinesterase, the tested fractions were found toxic at 1g/kg and onward by inducing vomiting, diarrhea, lethargy, and mortality. The dichloromethane fraction was found more emetic than the ethyl acetate fraction. It is also concluded that emesis caused by both the fractions was decreased by Metoclopramide (6mg/kg I.M) more than dimenhydrinate (6mg/kg). Hence, *Chrozophora tinctoria* has significant pharmacological effects and emetogenic activity.

Acknowledgements

The authors would like to thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work by Grant Code: 22UQU4350073DSR03.

References

ABDEL-NAIM, A.B., ALGHAMDI, A.A., ALGANDABY, M.M., AL-ABBASI, F.A., AL-ABD, A.M., EID, B.G., ABDALLAH, H.M. and EL-HALAWANY, A.M., 2018. Rutin isolated from *Chrozophora tinctoria* enhances bone cell proliferation and ossification marker. *Oxidative Medicine and Cellular Longevity*

AHMED, S., HASAN, M.M., AHMED, S.W., MAHMOOD, Z.A., AZHAR, I., and HABTEMARIAM, S., 2013. Anti-emetic effects of bioactive natural products. *Phytopharmacology*, vol. 4, no. 2, pp. 390-433.

AKHILA, J.S., SHYAMJITH, D. and ALWAR, M.C., 2007. Acute toxicity studies and determination of median lethal dose. *Current Science*, vol. 9, no. 7, pp. 917-920.

ALAM, M., UDDIN, G., SIDDIQUI, B.S., SADAT, A., KHAN, A.A. and RAUF, A., 2014. Gas chromatography-mass spectrometry (GC-MS) analysis, antimicrobial, phytotoxic, insecticidal and leishmanicidal activities of fixed oil from Viburnum

grandiflorum. *African Journal of Pharmacy and Pharmacology*, vol. 8, no. 46, pp. 1173-1179.

ANDREWS, P., 1992. Physiology of nausea and vomiting. *British Journal of Anaesthesia*, vol. 69, suppl. 1, pp. 2S-19S.

AYAZ, M., JUNAID, M., AHMED, J., ULLAH, F., SADIQ, A., AHMAD, S. and IMRAN, M., 2014. Phenolic contents, antioxidant and anticholinesterase potentials of crude extract, subsequent fractions and crude saponins from Polygonum hydropiper L. *BMC Complementary and Alternative Medicine*, vol. 14, no. 1, pp. 1-9. http://dx.doi.org/10.1186/1472-6882-14-145. PMid:24884823.

BAKHT, J., MUHAMMAD, T., ALI, H., ISLAM, A. and SHAFI, M., 2011. Effect of different solvent extracted sample of Allium sativum (Linn) on bacteria and fungi. *African Journal of Biotechnology*, vol. 10, no. 31, pp. 5910-5915.

BELAKHDAR, G., BENJOUAD, A. and ABDENNEBI, E., 2015. Determination of some bioactive chemical constituents from Thesium humile Vahl. *J Mater Environ Sci*, vol. 6, no. 10, pp. 2778-2783.

CHATTERJEE, S., KARMAKAR, A., AZMI, S.A. and BARIK, A. (2018). Antibacterial activity of long-chain primary alcohols from *Solena amplexicaulis* leaves. *Proceedings of the Zoological Society*, vol. 71, pp. 313-319. http://dx.doi.org/10.1007/s12595-017-0208-0.

DELAZAR, A., TALISCHI, B., NAZEMIYEH, H., REZAZADEH, H., NAHAR, L. and SARKER, S., 2006. Chrozophorin: a new acylated flavone glucoside from Chrozophora tinctoria (Euphorbiaceae). *Revista Brasileira de Farmacognosia*, vol. 16, no. 3, pp. 286-290. http://dx.doi.org/10.1590/S0102-695X2006000300002.

ELAIYARAJA, A. and CHANDRAMOHAN, G., 2018. Comparative phytochemical profile of crinum defixum Ker-Gawler leaves using GC-MS. *Journal of Drug Delivery and Therapeutics*, vol. 8, no. 4, pp. 365-380.

ELLMAN, G.L., COURTNEY, K.D., ANDRES JUNIOR, V. and FEATHERSTONE, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, vol. 7, no. 2, pp. 88-95. http://dx.doi.org/10.1016/0006-2952(61)90145-9. PMid:13726518.

ETKIN, N.L., 1997. Antimalarial plants used by hausa in Northern Nigeria. *Tropical Doctor*, vol. 27, no. 1, suppl. 1, pp. 12-16. http://dx.doi.org/10.1177/00494755970270S106. PMid:9204717.

GRALLA, R. J., OSOBA, D., KRIS, M. G., KIRKBRIDE, P., HESKETH, P. J., CHINNERY, L. W., CLARK-SNOW, R., GILL, D. P., GROSHEN, S., & GRUNBERG, S., 1999. Recommendations for the use of antiemetics: evidence-based, clinical practice guidelines. *Journal of Clinical Oncology*, vol. 17, no. 9, no. 2971-2994. http://dx.doi.org/10.1200/JCO.1999.17.9.2971.

HUSSAIN, M., RAZA, M., KHAN, R. and NAWAZ, S., 2015. In vivo assessment of antiemetic potential of crude extract of Vetiveria zizanioides (Linn); chick emesis model. *International Journal of Pharma Sciences*, vol. 5, no. 3, pp. 1068-1071.

JAMSHIDI-KIA, F., LORIGOOINI, Z. and AMINI-KHOEI, H., 2018. Medicinal plants: past history and future perspective. *Journal of Herbmed Pharmacology*, vol. 7, no. 1, pp. 1-7.

JOSHI, C.S., PRIYA, E.S. and VENKATARAMAN, S., 2007. Acute and subacute toxicity studies on the polyherbal antidiabetic formulation Diakyur in experimental animal models. *Journal of Health Science*, vol. 53, no. 2, pp. 245-249. http://dx.doi.org/10.1248/jhs.53.245.

KHAN, A., SULEMAN, M., ABDUL BAQI, S. and AYUB, M., 2019. 1. Phytochemicals and their role in curing fatal diseases: a review. *Pure and Applied Biology*, vol. 8, no. 1, pp. 343-354.

KHARE, C.P., 2007. '*Indian Medicinal Plants, an Illustrated Dictionary*.' Heidelberg, Springer Verlag http://dx.doi.org/10.1007/978-0-387-70638-2.

MACDOUGALL, M. R., & SHARMA, S., 2020. *Physiology, chemoreceptor trigger zone*. Treasure Island (FL): StatPearls Publishing.

MARZOUK, M.M., HUSSEIN, S.R., KASSEM, M.E., KAWASHTY, S.A. and EL NEGOUMY, S.I., 2016. Phytochemical constituents and chemosystematic significance of Chrozophora tinctoria (L.) Raf. *Natural Product Research*, vol. 30, no. 13, pp. 1537-1541. http://dx.doi.org/10.1080/14786419.2015.1045506. PMid:26119079.

MATHEW, M. and SUBRAMANIAN, S., 2014. In vitro screening for anti-cholinesterase and antioxidant activity of methanolic extracts of ayurvedic medicinal plants used for cognitive disorders. *PLoS One*, vol. 9, no. 1, pp. e86804. http://dx.doi.org/10.1371/journal.pone.0086804. PMid:24466247.

MUHAMMAD, N., ULLAH, S., ABU-IZNEID, T., RAUF, A., SHEHZAD, O., ATIF, M., KHAN, H., NAZ, H., HERRERA-CALDERON, O., KHALIL, A.A. and UDDIN, M.S., 2020. The pharmacological basis of Cuscuta reflexa whole plant as an antiemetic agent in pigeons. *Toxicology Reports*, vol. 7, pp. 1305-1310. http://dx.doi.org/10.1016/j.toxrep.2020.09.009. PMid:33024704.

MUKHERJEE, P.K., KUMAR, V., MAL, M. and HOUGHTON, P.J., 2007. Acetylcholinesterase inhibitors from plants. *Phytomedicine*, vol. 14, no. 4, pp. 289-300. http://dx.doi.org/10.1016/j.phymed.2007.02.002. PMid:17346955.

NYAMAI, D. W., ARIKA, W., OGOLA, P., NJAGI, E., & NGUGI, M., 2016. Medicinally important phytochemicals: an untapped research avenue. *Journal of Pharmacognosy and Phytochemistry*, vol. 4, no. 4, pp. 35-49.

RAMIREZ, K. and SHERIDAN, J.F., 2016. Antidepressant imipramine diminishes stress-induced inflammation in the periphery and central nervous system and related anxiety-and depressive-like behaviors. *Brain, Behavior, and Immunity*, vol. 57, pp. 293-303.

SANTANA, R.B.D., 2016. *Estudo restrospectivo de casos de gastrenterite hemorrágica em cães filhotes atendidos no HVET-UnB*, 51 p. Brasília: Universidade de Brasília. Trabalho de Conclusão de Curso de Graduação em Medicina Veterinária.

SHER, A., BAKHT, J. and KHEN, K., 2018. Antinociceptive, antipyretic and antimicrobial activities of Chrozophora tinctoria extracts. *Indian Journal of Pharmaceutical Sciences*, vol. 80, no. 3, pp. 533-540. http://dx.doi.org/10.4172/pharmaceutical-sciences.1000388.

SILBERNAGL, S., DESPOPOULOS, A., GAY, W.-R., ROTHENBURGER, A. and WANDREY, S.O.N., 2009. *Color atlas of physiology*. India: Thieme Stuttgart, vol. 5. http://dx.doi.org/10.1055/b-005-148939.

SNAFI, A.E.A., 2015. The chemical constituents and pharmacological importance of *Chrozophora tinctoria. International Journal of Pharmacy Review & Research*, vol. 5, no. 4, pp. 391-396.

SUDHA, T., CHIDAMBARAMPILLAI, S. and MOHAN, V., 2013. GC-MS analysis of bioactive components of aerial parts of Fluggea leucopyrus Willd. (Euphorbiaceae). *Journal of Applied Pharmaceutical Science*, vol. 3, no. 5, pp. 126-130. http://dx.doi.org/10.7324/JAPS.2013.3524.

TANIHATA, S., IGARASHI, H., SUZUKI, M. and UCHIYAMA, T., 2000. Cisplatin-induced early and delayed emesis in the pigeon. *British Journal of Pharmacology*, vol. 130, no. 1, pp. 132-138. http://dx.doi.org/10.1038/sj.bjp.0703283. PMid:10781008.

TAYADE, A.B., DHAR, P., KUMAR, J., SHARMA, M., CHAUHAN, R.S., CHAURASIA, O.P. and SRIVASTAVA, R.B., 2013. Chemometric profile of root extracts of Rhodiola imbricata Edgew. with hyphenated gas chromatography mass spectrometric technique. *PLoS One*, vol. 8, no. 1, pp. e52797. http://dx.doi.org/10.1371/journal.pone.0052797. PMid:23326358.

TYAGI, T., & AGARWAL, M., 2017. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of Pistia stratiotes L. and Eichhornia crassipes (Mart.) solms. *Journal of Pharmacognosy and Phytochemistry*, vol. 6, no. 1, pp. 195-206.