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IN VITRO DNA DAMAGE INHIBITION ACTIVITY OF AQUEOUS AND ETHYL ACETATE EXTRACT OF *VERNONIA AMYGDALINA* LEAVES

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ABSTRACT

Vernonia amygdalina (Asteraceae) is a plant widely used both for nutritional and medicinal purposes throughout tropical Africa. Its health-promoting ability might be related to the effect various bioactive components of the plant. In this study the DNA damage inhibition activity of this plant was investigated using the aqueous extract and the ethyl acetate fractions of the leaves. The fractions were used in various concentrations (10mg/ml, 5mg/ml and 1mg/ml) against DNA damage caused by Fenton's system and it was discovered that they protected the plasmid DNA from damage caused by the Fenton's system. The protective activity varied with concentration as the higher concentration showed better protective ability when compared to the lower concentration. The ethyl acetate fraction was subjected to Gas Chromatography Mass Spectrophotometry (GC-MS) analysis and fatty acids were separated from the fraction. These observations emphasize that both the aqueous and ethyl acetate extract of *V. amygdalina* possess DNA damage inhibition potential and although, the specific bioactive compound responsible for the inhibition activity was not discovered in this work the plant can be used to develop compounds for therapeutic uses.

KEYWORD: *Vernonia amygdalina*, Gc-Ms Extraction, DNA damage inhibition.

INTRODUCTION

Oxidation is a natural cellular metabolic process, which lead to the formation of free radicals commonly known as reactive oxygen species (ROS) or reactive nitrogen species (RNS), such as Superoxide, Hydroxyl, Hydrogen peroxide, Peroxyl radical, Ozone, Nitric oxide, Peroxynitrite, Peroxynitrous acid, Nitrogen dioxide free radicals. Now it is scientifically

proven that oxidative stress imposed by ROS and/or RNS can damage cellular membranes, proteins, fats and nucleic acid.[1] Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals commonly known as antioxidant defence mechanism. Failure of antioxidant defence mechanism to counter the free radicals plays an important role in many chronic and degenerative diseases, such as heart disease, cancer, diabetes mellitus, neurodegenerative diseases, Parkinson's disease, Alzheimer's disease and ageing.[2]

Antioxidants are the compounds with ability to neutralize free radicals, therefore prevent free radical mediated oxidative damage in cell. Antioxidant neutralize the free radicals by interfere with the oxidation process by reacting with free radicals, chelating activity, catalytic activity and oxygen scavenging activity.[3] Recently there has been a growing interest in the study of traditional plants for pharmaceutical applications because of its low toxicity and economic viability. In past, various plant phytochemicals viz phenolic compounds, flavonoids and tannins reported to possess significant antioxidant activity against a wide variety of free radicals.[4] These active compounds can be isolated and developed as natural drugs for prevention of many ailments.

Vernonia amygdalina, commonly known as bitter leaf, is a shrub that grows up to 3 meters high in the African tropics and other parts of Africa, particularly, Nigeria, Cameroon and Zimbabawe. It is reputed to have several health benefits. The organic fraction extracts of the plant was shown to possess cytotoxic effects towards human carcinoma cells of the nasopharynx.[5] It is effective against amoebic dysentery[6] gastrointestinal disorders[7] and has antimicrobial and antiparasitic activities antioxidant, hepatoprotective and chemoprotective activity.[9]

METHODOLOGY

SAMPLE COLLECTION

The plant leaves of *V. amygdalina* (bitter leaf) were collected from a vegetable garden at samaru market Zaria. The Plant was identified at the Herbarium of the Department of Biological Science, Ahmadu Bello University Zaria.

PREPARATION OF CURDE

The *V. amygdalina* leaves were shade dried for about five (5) days. The dried leaves were grinded into powdered form. 100ml of deionized water was added to the grinded *V.*

amygdalina and was allowed to stand for 24hours. It was filtered using filter papers and beakers and then it was allowed to dry in a water bath and was set at 50^{o}C and when dried was in a paste form.

PREPARATION OF FRACTION

50g of the crude sample was extracted in 300ml of ethyl acetate in glass conical flask on a shaker for 24h at room temperature. The extract was filtered with filter paper and dried on a water bath at 50-60 ^{0}C.

DNA DAMAGE ASSAY

The protective effect of *V. amygdalina* extracts were tested for deoxyribonucleic acid (DNA) damage based on the method of Lee *et al* (2002). with modification. The pVax plasmid DNA (476ng) was oxidized using Fenton's system (Fe^{3+}/ H_2O_2/ascorbic acid) in the presence or absence of *V. amygdalina* leaf extracts at (10mg/ml and 1mg/ml) and (10mg/ml, 5mg/ml and 1mg/ml) concentration for the crude extract and ethyl acetate extract respectively for 30 min at 37°C. After incubation of these compounds with DNA, 10µL of all the samples were loaded along with gel loading dye in 0.8% agarose gel for electrophoresis. The gel was scanned using the gel documentation system.[9]

GAS CHROMATOGRAPHY MASS SPECTROPHOTOMETRY (GCMS)

PRINCIPLE: Separation of volatile compounds in a mixture. The separated compounds can be identified and quantified; this can be achieved in a GC-MS system.

PROCEDURE

The ethyl acetate fraction was analyzed by Electron Impact Ionization (EI) method on GC-17A gas chromatograph, coupled to a GCMs 2010 plus mass spectrometer; fused silica capillary column temperature of 40˚ C (was held 2 min) was maintained with carrier gas helium at a constant pressure of 90kPa. Samples were injected by splitting with the split ratio10. Essential oil sample was dissolved in chloroform. The operating condition were as follows: name of column- RTS-5MS, diameter 30 cm, length 0.25mm, temperature of the column- initial temperature 40˚C (was held 2 min) , injector temperature- 220 ˚C, holding time 5 min, column packing column packing was done with 10% diethylene glycol succinate on 100-120 mesh diatomic CAW, splitting samples were injected by splitting with the spilt ratio 10, carrier gas- helium gas at constant pressure 90kPa, sample dissolved in chloroform, range of linear temperature increase 10˚C per min.[10]

RESULTS AND DISCUSSION

Protective effect of vernonia amygdalina leaves aqueous and ethyl acetate extract fraction against DNA damage

The *in vitro* DNA damage inhibition activity of the different leaf fractions of *V.amygdalina* was very active. Extracts were effective at varying concentrations of 10mg/ml and 1mg/ml for the aqueous fraction and 10mg/ml, 5mg/ml and 1mg/ml for the ethyl acetate fraction of the plant leaf.

From figure 1, the Agarose electrophoregram of the aqueous extract of *V. amygdalina* shows the DNA damage protecting activity, it protected plasmid DNA from free radicals generated by the Fenton's system and the protecting ability is in both concentrations of 10mg/ml and 1mg/ml (lane A and B) and the intensity of the band suggests the dependence of the protecting ability to concentrations at 10mg (lane A) and1mg (lane B) while lane C the positive control also revealed the presence of intact plasmids, lane D the negative control showed evidence of DNA damage by the Fenton's system.

From figure 2, the Agarose electrophoregram of the ethyl acetate extract of *V.amygdalina* also shows the protecting activity of the fraction, lane A shows the presence of the untreated plasmid DNA, lane B confirmed the damage of the plasmid DNA by the Fenton's system, while lane's D, E and F shows protective ability of the extract on the plasmid DNA against free radicals generated by the Fenton's system at 10mg/ml, 5mg/ml and 1mg/ml respectively.

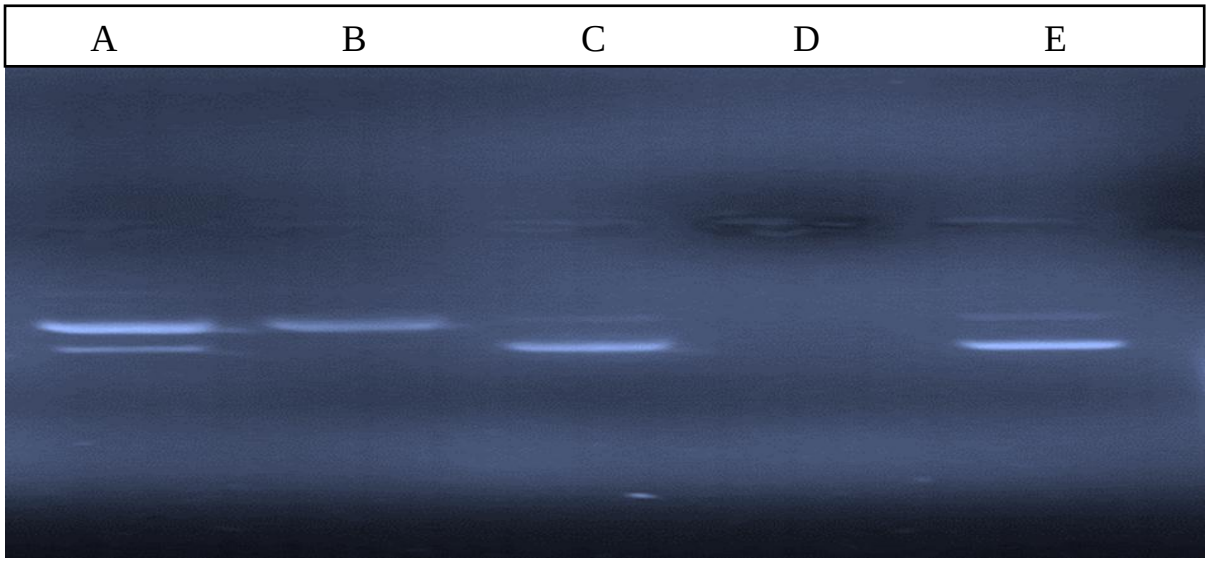

Figure 1: Effects of 10mg/ml and 1mg/ml of *V. Amygdalina* leaves aqueous extracts on the protection of plasmid DNA (pvax) against damage caused by fenton's system.
Keys: A= Plasmid + 10mg/ml of extract + Fenton's. **B**= Plasmid + 1mg/ml of extract + Fenton's. **C**= Untreated Plasmid. **D**= Plasmid + Fenton's. **E**= Plasmid + Extract.

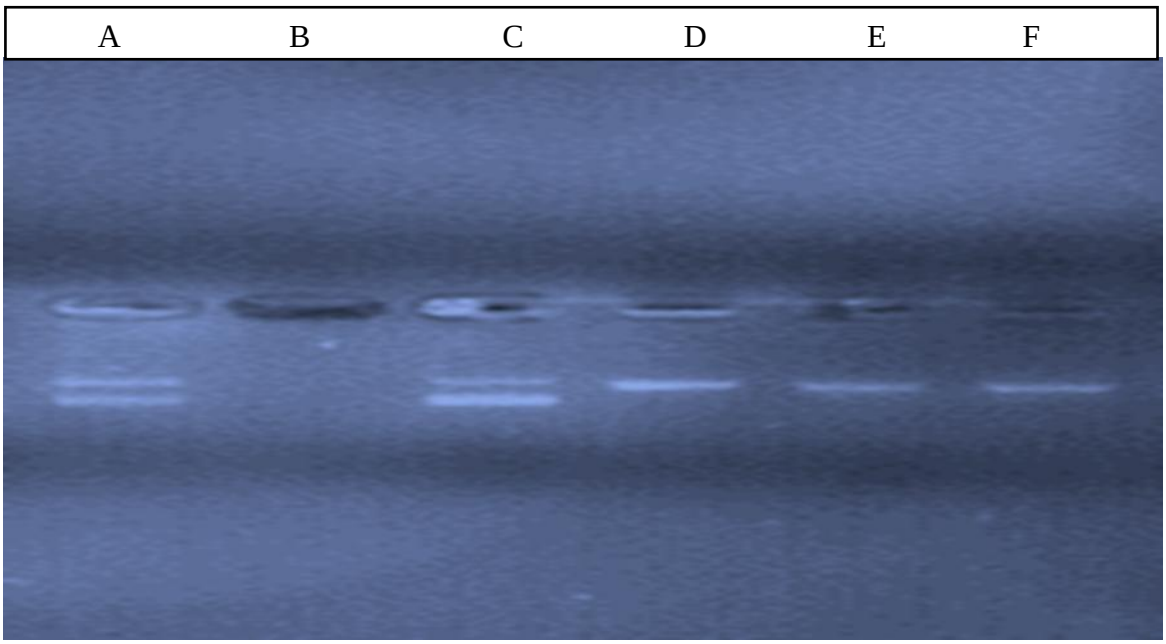

Figure 2: Effect of varying concentrations of *V. Amygdalina* leaves ethyl acetate extract on the protection of plasmid DNA (pvax) against damage caused by fenton's system.

Keys: A= Plasmid + extract + DMSO/PBS B= Plasmid + Fenton's C= Plasmid + DMSO/PBS D= Plasmid + 10mg/ml of extract + Fenton's E= Plasmid + 5mg/ml of extract + Fenton's E= Plasmid + 1mg/ml of extract + Fenton's.

Gc-Ms Analysis of ethyl acetate extract of *Vernonia Amygdalina*

The ethyl acetate fraction of *V.amygdalina* was subjected to GC-MS analysis to isolate and identify the bioactive compounds on the leaf extract.

Figure 3, shows the various peaks of compounds isolated. Which means about 19 compounds were separated. Table 2, reveals the area covered by each peak on the chromatogram in %, height of each peak retention time etc. Table 3, shows the compounds with the highest similarity index at each peak as compared to the GC-MS machine library with their molecular formulars and weights. The ethyl acetate fraction was characterized by the presence of aliphatic hydrocarbons (undecane, tetradecane), solvents, saturated fatty acids (Octadecanoic acid, n-Hexadecanoic acid).

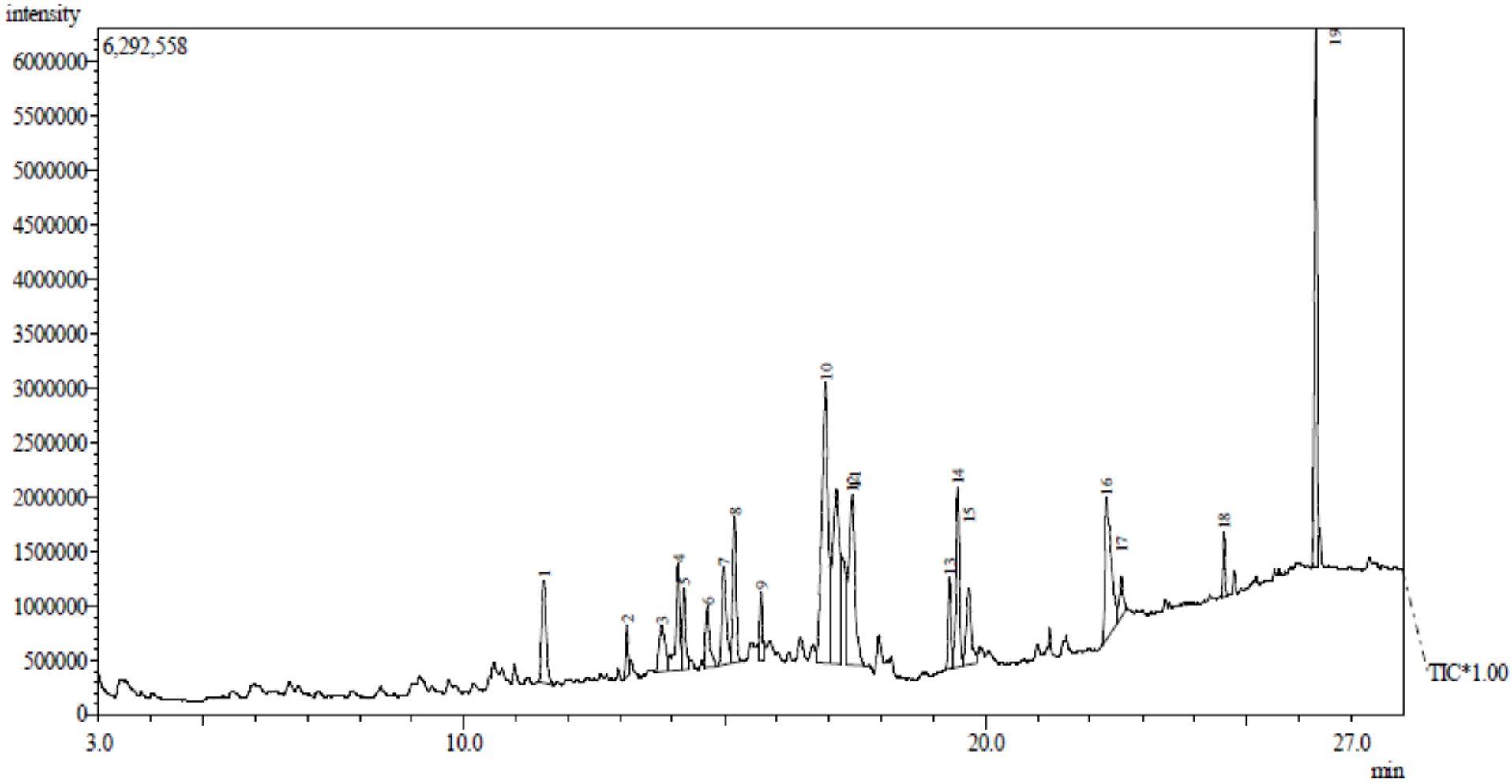

Figure 3: Chromatogram of Ethyl Acetate Extract of *V. Amygdalina* Showing the Various Peaks Of Compounds Separated.

Table 1: Peak report of the gas chromotography and mass spectrum.

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark
1	11.534	11.433	11.717	5526693	4.28	940558	4.07	5.88	
2	13.129	13.058	13.183	1535308	1.19	477199	2.06	3.22	
3	13.792	13.675	13.917	3472467	2.69	416863	1.80	8.33	
4	14.106	13.917	14.175	5018212	3.88	974156	4.21	5.15	V
5	14.221	14.175	14.325	2898205	2.24	750771	3.25	3.86	V
6	14.666	14.600	14.850	3009522	2.33	554300	2.40	5.43	V
7	14.981	14.850	15.100	5186437	4.01	896121	3.88	5.79	
8	15.187	15.100	15.292	5988866	4.63	1339421	5.79	4.47	V
9	15.695	15.650	15.742	1944228	1.50	625727	2.71	3.11	V
10	16.934	16.750	17.033	22361438	17.30	2575765	11.14	8.68	V
11	17.140	17.033	17.225	14375874	11.12	1607506	6.95	8.94	V
12	17.447	17.342	17.667	12631864	9.77	1560810	6.75	8.09	V
13	19.307	19.225	19.367	2937849	2.27	842859	3.65	3.49	
14	19.469	19.367	19.567	7698783	5.96	1648486	7.13	4.67	V
15	19.677	19.567	19.825	4637317	3.59	699251	3.02	6.63	V
16	22.313	22.217	22.525	10251828	7.93	1299047	5.62	7.89	
17	22.599	22.525	22.700	1908522	1.48	374624	1.62	5.09	V
18	24.565	24.517	24.633	1718929	1.33	599016	2.59	2.87	V
19	26.329	26.242	26.383	16155595	12.50	4933307	21.34	3.27	
				129257937	100.00	23115787	100.00		

Table 2: Compounds similar to the bioactive compound in the Gc-Ms machine library.

Peak	Retention Time	Area %	Molecular Formular	Molecular Weight	Similarity Index %	Compound
1	11.534	4.28	C10H18O2	170	88	2-Furanmethanol
2	13.129	1.19	C6H7NO2	125	81	2-Butenitriel
3	13.792	2.69	C9H16O2	156	71	2-Nonenoic
4	14.221	3.88	C10H20O2	172	80	7-Octane-2-6-diol
5	14.666	2.24	C10H18O2	170	82	2-7-octadiene-1-6-diol
6	14.981	2.33	C13H20O2	208	88	2-Cyclohexen-1-one
7	15.187	4.01	C10H16O2	168	87	7-Oxabicyclo(4.1.6)heptane
8	15.695	4.63	C10H14O2	166	88	5-7-Octadiene-2-one
9	16.943	1.50	C14H28	196	92	1-Tetradecene
10	16.934	17.30	C9H16O	140	84	Ethanone
11	17.140	11.12	C14H20O3	236	84	8-(2-Acetyloxiran-2-yl)-6
12	17.447	9.77	C11H18O2	182	79	2(1H)-Naphthalenone
13	19.307	2.27	C15H30	210	90	Tetracosarol
14	19.469	5.96	C16H22O4	278	80	1,2- Benzendicarboxylic acid
15	19.677	3.59	C16H32O2	256	92	n-Hexadecanoic
16	22.313	7.93	C20H40	280	86	Cyloeicosane
17	22.599	1.48	C18H36O2	284	82	Octadecanoic acid
18	24.565	1.33	C13H28	184	85	Undecane
19	26.329	12.50	C24H38O4	390	83	Di-n-octylphthalate

DISCUSSION

V. amygdalina is a tropical origin plant and widely distributed throughout Nigeria. *V. amygdalina* is plant with several traditional medicinal applications in traditional medication system. The plant was selected for this study based on its traditional medicinal uses to cure

malaria, nausea, diabetes, liver diseases, GIT disorders, kidney problems, antihelmints, bacterial infection etc.[11,12]

The protective effect of extracts on oxidative DNA strand breakage was evaluated with plasmid DNA. In this study both aqueous extract and ethyl acetate extract of *V. amygdalina* exhibited protection of plasmid DNA against free radicals mediated oxidative damage.[13] As seen in Fig 4 in the presence of Fenton's reagent as in lane D the plasmid DNA was totally degraded by the deleterious effect of free radicals generated by Fenton's system. Addition of the aqueous extracts as in lane A and B significantly protected the DNA when compared to the positive control in lane C. In Fig 2, the ethyl acetate extract treatment exhibited protection of the plasmid DNA at the varying concentrations (10mg/ml, 5mg/ml and 1mg/ml) as seen from lane D, E and F respectively. Moreover, a prominent damage was also observed in DNA treated with Fenton's system as seen in lane B.[13-15]

The result revealed that aqueous and ethyl acetate extract of *V. amygdalina* at both high and low concentration (10mg/ml and 1mg/ml) were potent in preventing oxidative damage on the DNA. Moreover, *V. amygdalina* leaf extracts showed higher protective effect as the concentration increased as seen in lane A and B of (Fig 4) and lane D, E and F of (Fig 2) 10mg/ml showed a thicker band than that of 5mg/ml and 1mg/ml concentration of the extracts.

The GC–MS analysis of the ethyl acetate extract from the leaves of *V. amygdalina* revealed the presence of some fatty acids, aliphatic hydrocarbons and solvents. palmitic acid, myristic acid and steric acid were the most abundant lipid in the extract (Table 1). *V. amygdalina* is known to have abundant fatty acids (saturated and unsaturated).[15,16] α-linolenic and linoleic acid are the two important fatty acids in *V. amygdalina* leaf. These lipids have both therapeutic and nutritional importance to humans. In contrast to the work done on the fatty acid assay of *V. amygdalina* leaf extract,[17] in this study only saturated fatty acids were separated after the GC-MS analysis. According to the World Health Organization (WHO) palmitic acid has antimutagenic activity and little amount of antioxidant property.[18] The identified saturated lipids myristic acid and palmitic acid have been reported to elevate blood cholesterol while stearic acid has demonstrated a good ability to lower cholesterol by 21 %.[19-22]

CONCLUSION

Antioxidant-rich plant extracts serve as nutraceuticals that alleviate the oxidative stress and therefore slow down the degenerative diseases. This work reveals that *V. amygdalina;* aqueous and ethyl acetate extract has an effect on the action of free radicals and reactive species against the integrity of the DNA. These effects were observed to be dependent on the concentration, which reveals that the higher the concentration of the leaf extract, the more effective or more pronounced the protective activity would be. Although, saturated fatty acids were obtained from the GC-MS analysis and palmitic acid is said to have antimutagenic and antioxidant properties, it's the unsaturated fatty acids in the leaf that are responsible for most of its antioxidant, immunological and medicinal benefits.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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