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1 # Elemental analysis of *Moringa oleifera* seeds by Laser Induced Breakdown
2 Spectroscopy (LIBS) and its anti-cancer and anti-microbial studies

3

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[1] ***Abbreviations:*** MOSs, *Moringa oleifera* seed; LIBS, Laser Induced Breakdown Spectroscopy; GC-MS ,Gas chromatography–mass spectrometry.

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19 **Abstract**

20 Moringa oleifera plant contains a numerous antioxidants, antibiotics and nutrients (vitamins and minerals)
21 which makes it prospective for diverse biomedical applications. This report investigated the anti-
22 cancerous and anti-microbial potential of the Moringa oleifera seeds (MOSs) due to the bioactive
23 components a detail elemental analysis. MOSs in the form of pellets were used in elemental analysis.
24 Alcoholic extraction of were utilized in anti-cancerous and anti-microbial activity.To clarify the anti-
25 cancerous and anti-microbial potential of the (MOSs) because of the bioactive components. An elemental
26 analysis was conducted using Laser Induced Breakdown Spectroscopy (LIBS) . The GC-MS used for the
27 validation of the LIBS outcome. Additionally, the anti-cancerous and anti-microbial activity of the MOSs
28 was evaluated. Herein, the human colorectal carcinoma cells (HCT-116) were treated with the seeds
29 aqueous extracts for 48 h and the cell viability plus the DNA nuclear morphology were measured via the
30 MTT assay and DAPI staining. The cell viability of the normal human embryonic stem cells (HEK-293)
31 was also examined after the treatment. Percentage of cell viability and inhibitory concentration (IC_{50}) of
32 both normal and treated cells were determined.The recorded LIBS signal of the MOSs revealed the
33 existence of elements like Ca, K, Mg, P, S, Fe, Mn, Zn, Na and Se (vital for human health). The results of
34 the MTT assay revealed a profound inhibitory action of the MOSs extracts against the HCT-116 cells
35 growth. On top, such extracts did not affect the HEK-293 cells growth, indicating the specificity of the
36 proposed extracts towards cancer cells escalation hindrance. The anti-microbial activity of the ethanolic
37 MOSs extracts was tested on the S. aureus and E. coli bacteria using the Agar well diffusion assay. The
38 observed anti-cancerous and the anti-microbial activities of the MOSs extracts can be attributed to the
39 charisma of various bioactive compounds including the oleic acid, palmitic acid, hexadecenoic acid ethyl
40 ester and D-allose.

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41 *Conclusion:* Current observations may contribute towards the development of the MOSs-based
42 biomedicine (organic without any side effects, cheap, plentiful, pure and sustainable) effective for the
43 cancer and bacterial infection cure.

44 **Keywords:** *Moringa oleifera;* seed extracts; LIBS; GC-MS ;anti-cancer; anti-microbial

45

46 1. Introduction

47 Generally, the moringa tree with the scientific name of Moringa oleifera Lam. (MOL) is the part of
48 Moringaceae family [1] . Being originated from the Northern India and Pakistan region alongside the
49 Himalaya Mountains, moringa tree also grows in different parts of African, Asian, and South American
50 tropics [2-4] . In Pakistan, India, Thailand and Philippines the moringa leaves and fruits are usually
51 consumed as the vegetables [5]. In recent times, several countries (Mexico, Hawaii, Cambodia, and the
52 Caribbean Islands) started cultivating it due to its numerous health benefits and nutritional values [6,7].
53 Apart from the staple organic food with enriched vitamins, proteins and minerals, the natural moringa
54 leaves and fruits have several biological applications [5,8]. The MO as a food is recommended by the
55 WHO due to its ramified nutritionals benefits to human health [5].

56 In the past, several emperors worldwide used to consume the fruits and leaves of the Moringa plants
57 for keeping the skin healthy, acquiring energy, relieving the tension/stress and pain during the battles [8]
58 . Intensive studies revealed that the MO have many notable attributes including anti-oxidant, anti-
59 microbial, anti-cancer, anti-inflammatory, anti-ulcer, anti-hypertensive, anti-urolithiatic, anti-diabetic,
60 anti-asthmatic, anti-aging, cardiovascular, analgesic, immunomodulation, hepatoprotective, diuretic,
61 anthelmintic, and hypoglycemic characteristics [2,9-19]. In fact, the presence of some distinctive
62 chemical compounds and elements such as the 4-(4′-O-acetyl-α-L-rhamnopyranosyloxy) benzyl
63 isothiocyanate, niazimicin, 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate, pterygospermin, 4-(α-

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64 Lrhamnopyranosyloxy), benzyl isothiocyanate, and benzyl glucosinolate impart the hypo-tensive, anti-
65 bacterial and anti-cancer efficacies to the MO leaves and fruits [20]

66 To take the advantages of the MO plant products as the food supplements and medicines as well as
67 to determine its other health benefits various techniques have been developed for extracting the contents
68 from the MO leaves and fruits. These methods include pressurized liquid extraction [21], ultrasound [22],
69 microwave-based extraction [23], and supercritical fluid extraction [24]. However, all these techniques
70 suffer from some limitations in terms of the requirement of a large quantity of toxic organic solvents,
71 cumbersome sample preparation procedures and costly [25]. To overcome such drawbacks the LIBS has
72 been emerged as an efficient approach to determine the elemental compositions (various trace elements)
73 in the extracts obtained from different medicinal plants.

74 Currently, the laser induced breakdown spectroscopy (LIBS) technique has widely been exploited
75 for elemental analyses of different types of samples. It is a versatile analytical technique with many distinct
76 features such as the real-time measurement, rapidity, cost-effectiveness, sensitiveness, and in-situ
77 elemental analysis with microanalyses capacity for diverse types of materials and phases (solid, liquid, or
78 gas) [26-31]. In addition, this technique is simple, eco-friendly, and does not need cumbersome sample
79 preparation protocol because the sample treatments frequently tend to induce errors through contamination
80 and losses. Depending on the varieties of the inorganic constituents present in the medicinal plants, the
81 LIBS technique can accurately disclose their secrecy in a scientific manner. It is needless to mention that,
82 besides the occurrences of diverse organic molecular components most of the edible plants (herbs and
83 vines) are enriched with inorganic molecules and elements (in the form of vitamins, proteins and
84 minerals), signifying their immense health benefits. Since ancient times, the Ayurvedic medicinal
85 practices have been relied on these crude chemical components extracted from the medicinal plants

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86 [32,33]. The screening of the glycemic index of various elements in diverse medicinal plants was
87 performed to evaluate their anti-diabetic potency [34,35].
88 Based on the abovementioned factors, for the first time we used the LIBS technique to analyze the
89 presence of various trace elements in the MO seeds (MOSs) extracts. The gas chromatography - mass
90 spectroscopy (GC-MS) measurement of the ethanolic MOSs extract was carried out to validate the LIBS
91 results. In addition, the anti-cancerous and anti-microbial characteristics of the MOSs extracts were
92 evaluated. The human colorectal carcinoma (HCT-116) cancerous cells and embryonic kidney (HEK-293)
93 normal cells were chosen for the anti-cancerous assessment (using MTT assays) of the MOSs extracts.
94 The *gram-positive Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) bacterial strains were
95 selected for the bactericidal evaluation (using Agar well diffusion) of the MOSs extracts. Results were
96 interpreted and discussed to understand the mechanism of the anti-cancerous and anti-microbial
97 effectiveness of such extracts.
98

99 **2. Experimental procedure**

100 2.1. Seeds collection and extract synthesis

101 The good quality MOSs were purchased from the local market (originally imported from India) and
102 used for the extract preparation in few steps. The coatings of the seeds were first removed before being
103 crushed and ground to fine powder. Next, the resultant powder was compressed in the form of pellets.
104 Fig.1 (a)-(d) shows various stages of the sample preparation for further analyses using the LIBS technique.
105 Various amounts of aliquots (5, 10, 15, 20 and 25 g) were mixed separately in 200 ml of deionized water
106 (DIW). The obtained solution was heated at 60 ˚C with constant stirring and then kept overnight before
107 being filtered (Whatman filter paper No.1, Sigma-Aldrich). The obtained filtrate was maintained at 4 °C
108 for the anti-cancerous activity analyses of the MOSs. An alcoholic extraction was prepared for the

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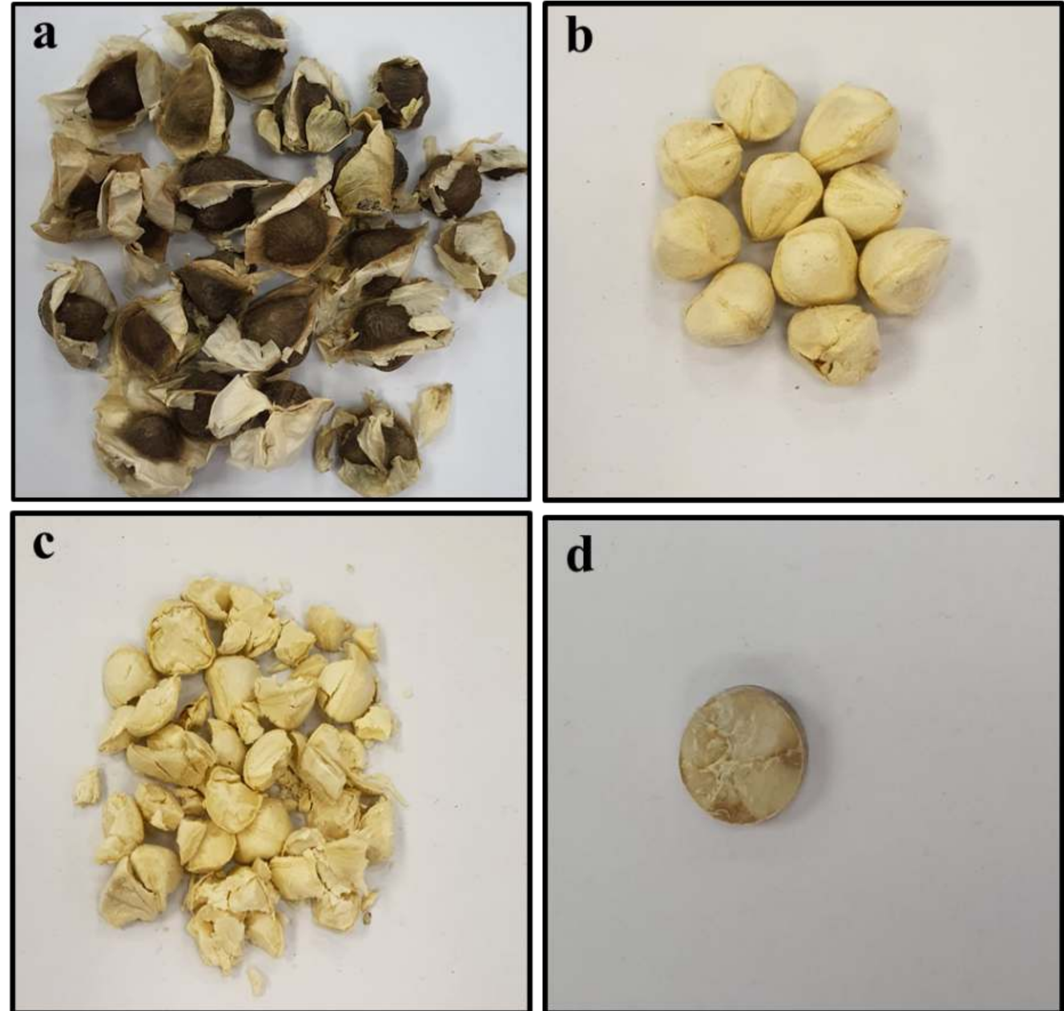
109 antimicrobial activity test. In this process, the fine powder of MOSs of various contents (5, 10, 15, 20 and
110 25 gm) was mixed in ethanol (200 ml) separately and stirred for 5 h. Later, the solutions containing the
111 MOSs were filtered and placed inside a rotary evaporator.
112
113



114
115 **Fig 1**. *Moringa oleifera* seeds sample: **a)** seed, **b)** seeds without coat, **c)** crushed seeds, **d)** seeds in the form
116 of pellets.
117

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118 2.2. LIBS Setup Details

119 Fig 2 depicts the customized LIBS setup used to quantify the constituent elements present in the
120 MOSs samples. It consisted of quadrupled Q-switched Nd:YAG laser (model QUV-266-5) operated at the
121 wavelength of 266 nm, repetition rate of 20 Hz, maximum output energy of 30 mJ and pulse width of 8
122 ns. The collimated laser pulses were focused onto the MOSs pellet (target) using a UV convex lens of 30
123 mm focal length for ablation. Upon ablating the target pellets a plasma plume was generated. The emitted
124 plasma was detected/collected using the fiber optical system equipped with a miniaturized lens placed at
125 an angle of 45°. The other end of fiber was connected to the 500 mm spectrograph (Andor SR 500i-A)
126 with the grating groove of approximately 1200 lines/mm. The pellet was mounted on a 2-D motorized
127 sample holder capable of moving in the X-Y direction to avoid the crust formation on the target surface
128 due to multiple laser shots on the same spot. The emission spectra were recorded by a ICCD camera
129 (model iStar 320T, 690 X 255 pixels with delay time setting at 300 ns) and transferred to the interfaced
130 on line personal computer (PC).

131

132

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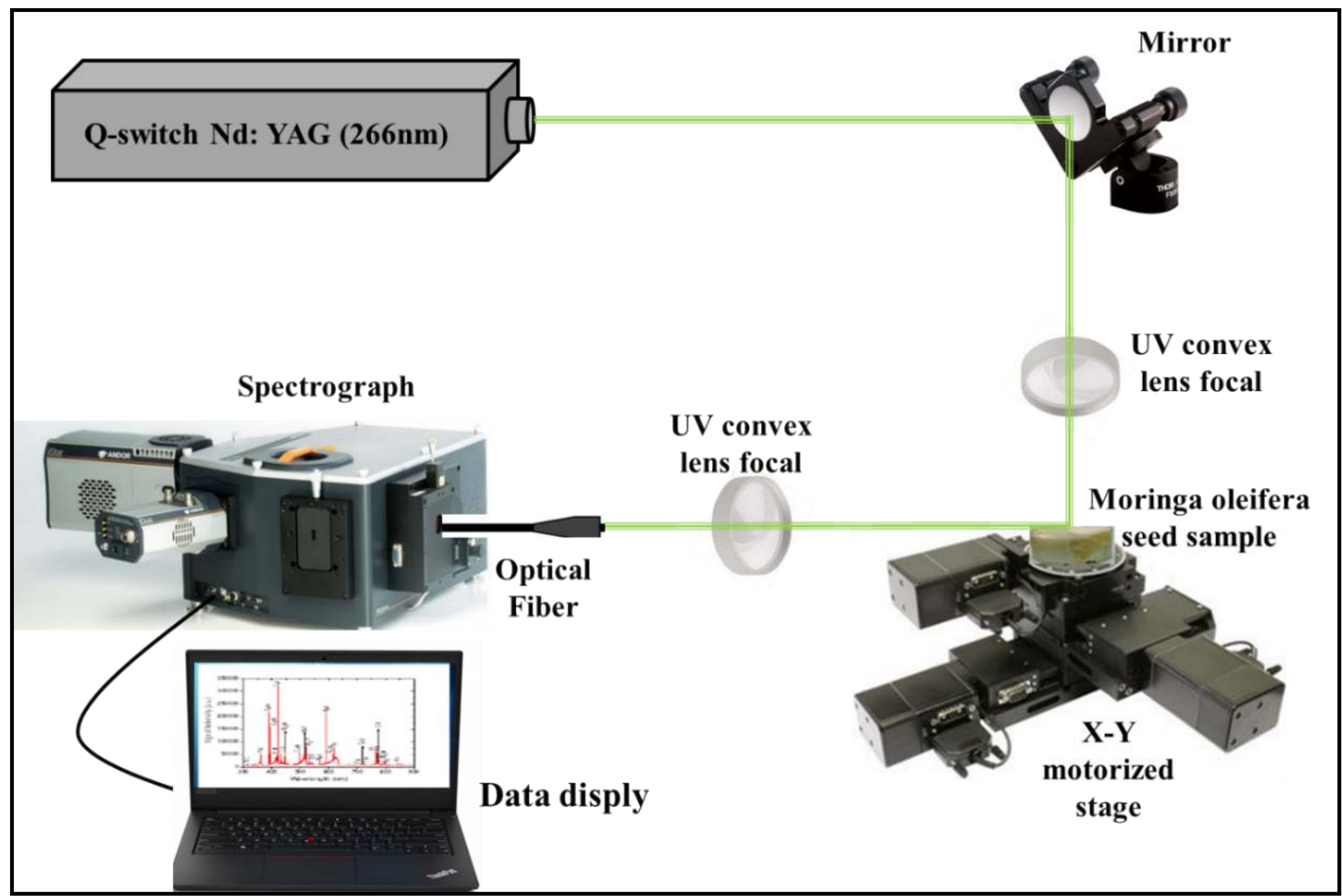

133

134 **Fig. 2**. Schematic of our LIBS setup, where the inset shows an image of *Moringa oleifera* seed sample.

135

136

137 2.3 GC-MS measurement

138 The ethanolic MOSs extract was analyzed using GC-MS (Shimadzu GC-2010 Plus) which was
139 equipped with a split/spitless auto-injector (AOC-20i series) and coupled to a QP2010 Ultra single
140 quadrupole (Shimadzu Corporation, Kyoto, Japan). The GC separation was achieved via an Rxi-5MS
141 fused silica capillary column (Restek, USA) of dimension (30 m × 0.25 mm id × 100 µm film thickness).
142 The temperature was raised from 60 °C (kept for 0.5 min) to 280 °C at the rate of 5 °C/min (hold for 5
143 min). The inlet was operated in the splitless mode at the temperature of 270 °C. Helium was flown (purity

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144 of 99.9999%) as the carrier gas at the rate of 1 ml/min and the temperature of the MS transfer line was
145 maintained at 280 °C. The ion source was worked in the electron impact mode at the energy of 70 eV and
146 temperature of 250 °C. The full scan mass spectra were collected from 33 to 550 m/z. The spectral data
147 was obtained by controlling the GC-MS and processed using the GC-MS solution (version 4.52, Shimadzu
148 Corporation, Japan). The detected volatile compounds were identified using the NIST 11 and WILEY 9
149 libraries and relative area of each compound was calculated.

150

151 2.4. Anti-cancerous activity evaluation of MOSs extract

152 2.4.1. In vitro cell culture and cell viability test

153 In this study, the cancerous and normal (healthy) cell line including the respective human colorectal
154 carcinoma (HCT-116) and embryonic kidney (HEK-293) cells were used to evaluate the anti-cancerous
155 activity of various MOSs extracts on these cells. Following the earlier prescribed method [36,37], the
156 proposed cells were seeded in 96 well plates and grown in the Dulbecco's Modified
157 Eagle Medium (DMEM) supplemented with the reagents such as selenium chloride, L-glutamine, fetal
158 bovine serum, antibiotic penicillin and streptomycin. One group of these cells were cultured inside a CO_2
159 incubator at the temperature of 37 °C and treated at various concentrations (0.030 mg/ml to 0.1 mg/ml) of
160 the MOSs extracts. Another group was treated at different concentrations (0.030 mg/ml to 0.1 mg/ml) of
161 the MOSs extracts. The control group was devoid of the MOSs extracts. After treating the cells using the
162 MOSs extracts for 48 hrs they were treated with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium
163 bromide (MTT assay, Molecules, New Zealand) for 4 hrs. Next, the growth medium was eliminated from
164 the plates and dimethyl sulfoxide (DMSO) was incorporated in every well where MTT created the
165 Formazan crystals. Later, the culture plates were inspected at the wavelength of 570 nm using a microplate

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166 reader (Bio-Rad Laboratories, Hercules, CA, USA). Finally, the GraphPad Prism Software was used to
167 analyze the acquired data where a one-way analysis of variance (ANOVA) statistical tool was utilized.
168
169 2.4.2. Nuclear staining via DAPI
170 The cancer cells (HCT-116) were cultured inside the CO_2 incubator at 37 °C and treated for 48 hrs
171 using the MOSs extract of concentration 0.066 mg/ml. The control group was made without adding any
172 such extract. The effects of the MOSs extracts on the cells nuclei were studied after staining by DAPI.
173 Next, the cold paraformaldehyde (4%) was used to pre-treat these cells and washed using Triton X-100
174 (0.1%) prepared in the phosphate buffer saline (PBS). Then, both control cell and those treated with the
175 MOSs extracts were stained using DAPI (1.0 µg/ml) made in the PBS. Lastly, the cells were rinsed using
176 Triton X-100 (0.1%) made in PBS. The morphologies of the control cells and those treated by MOSs
177 extracts were scanned via a confocal scanning microscope (CSM, Zeiss, Germany).
178
179 2.5. Anti-microbial activity assessment of MOSs extract
180 The bactericidal efficacy of the proposed MOSs extracts at various concentrations (50, 100, 150, 200,
181 250 mg/ml) was assessed using the Agar well diffusion method. The *gram-positive S. aureus* and *E. coli*
182 bacterial strain were selected. For the preparation of inoculum, bacteria were freshly grown in the nutrient
183 broth (NB) at 37 °C by incubating overnight and regulated to the 0.5 McFarland standards. The Mueller Hinton
184 agar solution was prepared by adding 100 µl of freshly adjusted inoculum. The inoculum was spread uniformly
185 over the surface of the MHA plates and left to dry under the aseptic conditions. Consequently, the sterile cork-
186 borer was used to punch the inoculated plates for the wells of 6 mm. Approximately 50 µl of the prepared extract
187 was placed in the wells at 37 °C for overnight incubation. At the end of the incubation period, the bacterial zone

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188 of inhibition (ZOI) diameter around the wells was measured to evaluate the inhibition effectiveness of the
189 studied MOSs extracts [38].
190

191 3. Results and Discussion

192 3.1. Qualitative analysis of MOSs using LIBS

193 The LIBS spectra (Fig 3) of the MOSs were recorded in range of 200 to 800 nm. For detecting the
194 major elements, the spectra were collected from different regions of the pellets each of scan length 50 nm.
195 In order to reduce the background noise that may affect the quality of the LIBS signal, the LIBS parameters
196 including the delay times, number of accumulations and laser energies were optimized. The recorded LIBS
197 signal of the MOSs comprised of various significant spectral peaks of the atomic and ionic lines (with
198 varying intensities) were related to abundant elements. Based on the NIST database the observed spectral
199 lines were identified and categorized in terms of the characteristic elements present in the MOSs,
200 suggesting their significant role towards the anti-microbial and anti-cancerous activities. Clearly, the
201 measured LIBS spectra (Fig 3) showed the presence of Ca, K, Mg, P, S, Fe, Mn, Zn, Na and Se.
202

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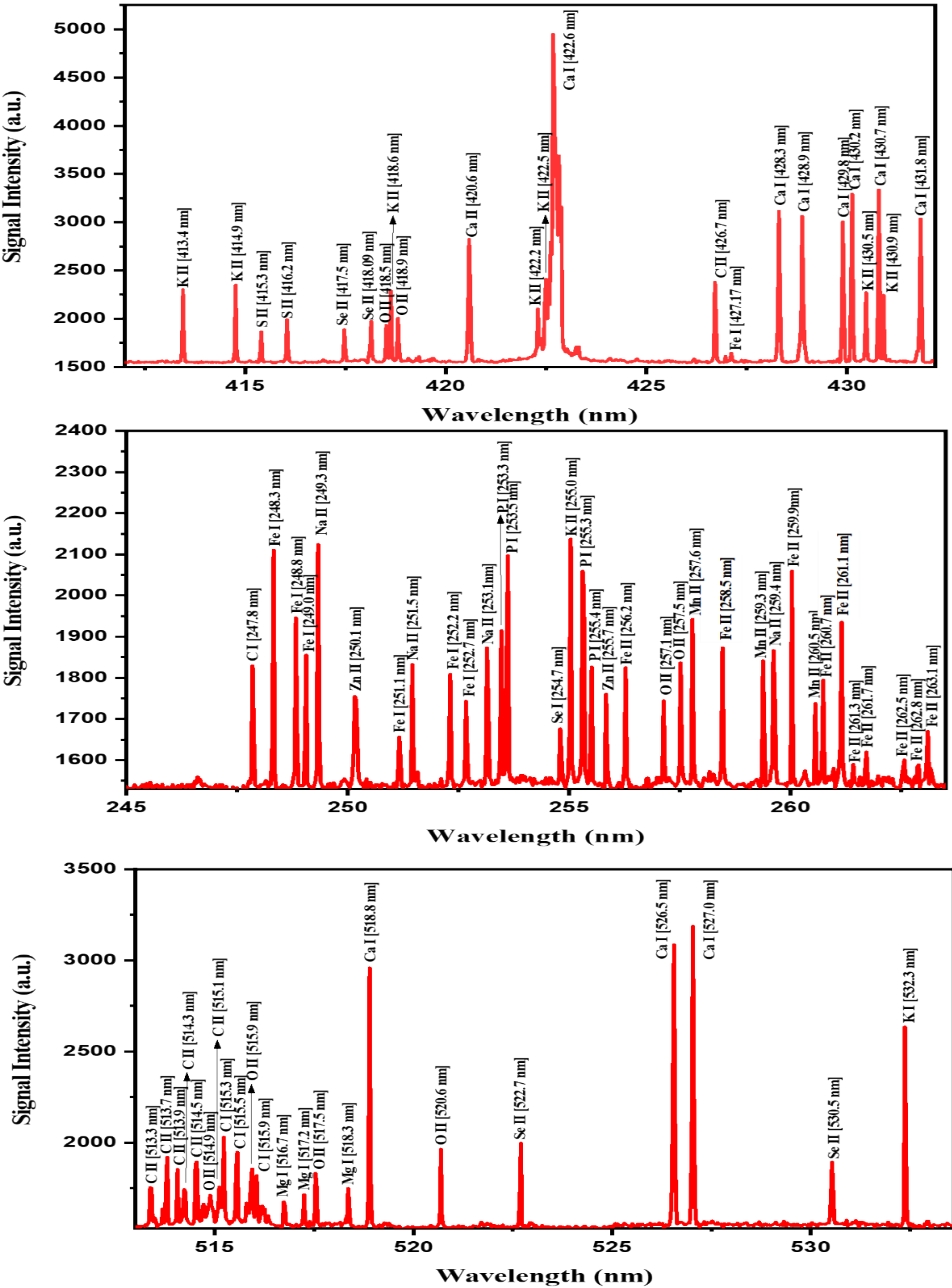

203

204 **Fig 3.** LIBS spectra of *Moringa oleifera* seeds.

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205

206 Table 1 presents the LIBS signal intensities of the spectral transition lines corresponding to various
207 detected elements in MOSs. In accordance to the Boltzmann distribution, the intensities of the LIBS
208 spectral lines have direct relationship with elemental contents in MOSs [39] . This correlation was attained
209 by considering the intensity ratio of the detected elemental lines with that of the C line taken as the
210 reference (247.8 nm). The achieved intensity ratio of the elements Ca, K, Mg, P, S, Fe, Mn, Zn, Na and
211 Se were ranged from 2.7 - 1.7, 1.2 - 1.4, 0.9, 1.1, 1.1, 1.1 - 0.9, 1.0, 0.9, 1.2 and 1.1, respectively which
212 were consistent with those reported in the literature [40]. These results approved that the MOSs are rich
213 in different minerals which are useful to human as food and medicine, indicating their remarkable
214 influence in regulating the level of blood pressure, blood lipids, regulating the stomach, protecting the
215 liver, strengthening bones, generate protein and enhancing the immunity of the body [41] . Furthermore,
216 the existence of Se in the MOSs seeds plays a vital role by protecting from cancer, cardiovascular disease,
217 cognitive decline and thyroid disease. Moreover, these seeds exhibit a powerful antibacterial activity due
218 to the availability of the detected elements including Ca, K, Mg, P, S, Fe, Mn, Zn, Na and Se [42].

219

220 **Table 1**. Some of the detected spectral lines of the elemental analytes present in the Moringa seeds
221 recorded by using our LIBS system.

Elements	Wavelength (nm)	Transition Configuration	LIBS Signal Intensity (a.u.)
Ca	422.6	$3p^6 4s^2\ ^1S_0 \rightarrow 3p^6 4s4p\ ^1P^\circ_1$	4960.9
	518.8	$3p^6 4s4p\ ^1P^\circ_1 \rightarrow 3p^6 4s5d\ ^1D_2$	2967.1
	527.0	$3p^6 3d4s\ ^3D_3 \rightarrow 3p^6 3d4p\ ^3P^\circ_2$	3196.3

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Fe	248.3	$3d^6\ 4s^2\ {}^5D_4 \rightarrow 3d^6({}^5D)4s4p\ ({}^1P^o)\ {}^5F^\circ_5$	2114.1
	252.2	$3d^6\ 4s^2\ {}^5D_4 \rightarrow 3d^6({}^5D)\ 4s4p\ ({}^1P^\circ)\ {}^5D^o_4$	1810.1
	259. 9	$3d^6\ ({}^5D)\ 4s\ {}^6D_{9/2} \rightarrow 3d^6({}^5D)\ 4p\ {}^6D^o_{9/2}$	2061.9
K	414.9	$3p^5\ 3d\ {}^3P^\circ_0 \rightarrow 3p^5\ 4p\ {}^3D_1$	2347.6
	430.5	$3p^5\ 3d\ {}^3P^\circ_2 \rightarrow 3p^5\ 4p\ {}^1D_2$	2270.9
	532.3	$3p^6\ 4p\ {}^2P^\circ_{1/2} \rightarrow 3p^6\ 8s\ {}^2S_{1/2}$	2639.7
Mg	518.3	$3s3p\ {}^3P^o_2 \rightarrow 3s4s\ {}^3S_1$	1753.5
Mn	259.3	$3d^5\ ({}^6S)\ 4s\ {}^7S_3 \rightarrow 3d^5({}^6S)\ 4p\ ({}^7P^\circ_3)$	1843.9
Na	249.3	$2s^2\ 2p^5\ 3s\ {}^1P^o_1 \rightarrow 2s^2\ 2p^5\ 3p\ {}^1S_0$	2128.4
	251.5	$2s^2\ 2p^5\ 3p\ {}^3S_1 \rightarrow 2s^2\ 2p^5\ ({}^2P^\circ_{1/2})\ 3d\ {}^2[{}^3/_2]^o_2$	1834.7
P	253.5	$3s^2 3p^3\ {}^2P^o_{3/2} \rightarrow 3s^2 3p^2({}^3P)\ 4s\ {}^2P_{3/2}$	2100.0
	255.3	$3s^2 3p^3\ {}^2P^o_{1/2} \rightarrow 3s^2 3p^2({}^3P)\ 4s\ {}^2P_{1/2}$	2032.1
S	416.2	$3s^2 3p^2({}^3P)\ 4p\ {}^4D^o_{7/2} \rightarrow 3s^2 3p^2({}^3P)\ 4d\ {}^4F_{9/2}$	1987.8
Se	418.09	$4s^2\ 4p^2\ ({}^3P)\ 5p\ {}^4D^o_{7/2} \rightarrow 4s^2\ 4p^2\ ({}^3P)\ 5d\ {}^4F_{9/2}$	1969.7
	522.7	$4s^2\ 4p^2\ ({}^3P)\ 5s\ {}^4P_{5/2} \rightarrow 4s^2\ 4p^2\ ({}^3P)\ 5p\ {}^4D^o_{7/2}$	2002.0
Zn	250.1	$3d^{10}4p\ {}^2P^o_{1/2} \rightarrow 3d^{10}\ 5s\ {}^2S_{1/2}$	1751.4
	255.7	$3d^{10}4p\ {}^2P^o_{3/2} \rightarrow 3d^{10}\ 5s\ {}^2S_{1/2}$	1762.3
C	426.7	$2s^2\ 3d\ {}^2D_{5/2} \rightarrow 2s^2\ 4f\ {}^2F^o_{7/2}$	2379.0
	247.8	$2s^2\ 2p^2\ {}^1S_0 \rightarrow 2s^2\ 2p3s\ {}^1P^o_1$	1831.6
O	418.9	$2s^2\ 2p^2\ ({}^1D)\ 3p\ {}^2F^o_{7/2} \rightarrow 2s^2\ 2p^2\ ({}^1D)\ 3d\ {}^2G_{9/2}$	2003.4

3.2. Volatile content analyses of MOSs using GC-MS

A total of 114 volatile compounds were detected in the proposed MOSs via GC-MS data analyses. These compounds included different chemical groups including varieties of fatty acid, aldehyde, ester,

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243 alcohol, ketone and hydrocarbon. Table 2 and Fig 4 show the retention times and percentage composition
244 of all the identified chemical compounds existed in the MOSs. The detected major constituents were the
245 oleic acid (22.53%), 2,3-dihydroxypropyl elaidate (13.48%), 9-octadecenoic acid (Z)-, 2,3-
246 dihydroxypropyl ester (11.35%), docosenamide (6.04%), ethyl oleate (6.03%), 1,3-propanediol, 2-ethyl-
247 2-(hydroxymethyl) (5.52%), oleic anhydride (3.96%) and 2-Propanone, 1,1-dimethoxy (3.86%). These
248 MOSs are rich in the fatty acids and their ester derivatives (65.45%) followed by alcohols (9.4%), nitrogen
249 containing compounds (9.09%), ketones (5.34%) and aldehydes (2.88%). The fatty acids and alcohols in
250 plants may undergo esterification to form esters [43]. The GC-MS measurement revealed the occurrences
251 of several fatty acids and their esters such as oleic, n-hexadecenoic (palmitic), cis-9-Hexadecenoic acid
252 (Palmitoleic), octadecanoic (stearic) acids and their alcohols and esters. In fact, most of such compounds
253 have been reported to possess anti-cancer activity. For example, the D-allose was reported to inhibit cancer
254 cells growth at G1 phase [44]. The palmitic acid disclosed a selective cytotoxicity against the leukemic
255 cells in humans [45] . In addition, such fatty acids are known to have antifungal and antibacterial effects
256 [46]. The oleic acid that was identified as the major compound in the MOSs is an omega-9-fatty acid with
257 numerous benefits for the human health. This can effectively be used to prevent the ulcerative colitis [47]
258 and reduce the blood pressure [48] . On top, it has remarkable antioxidant efficiency [49].

259

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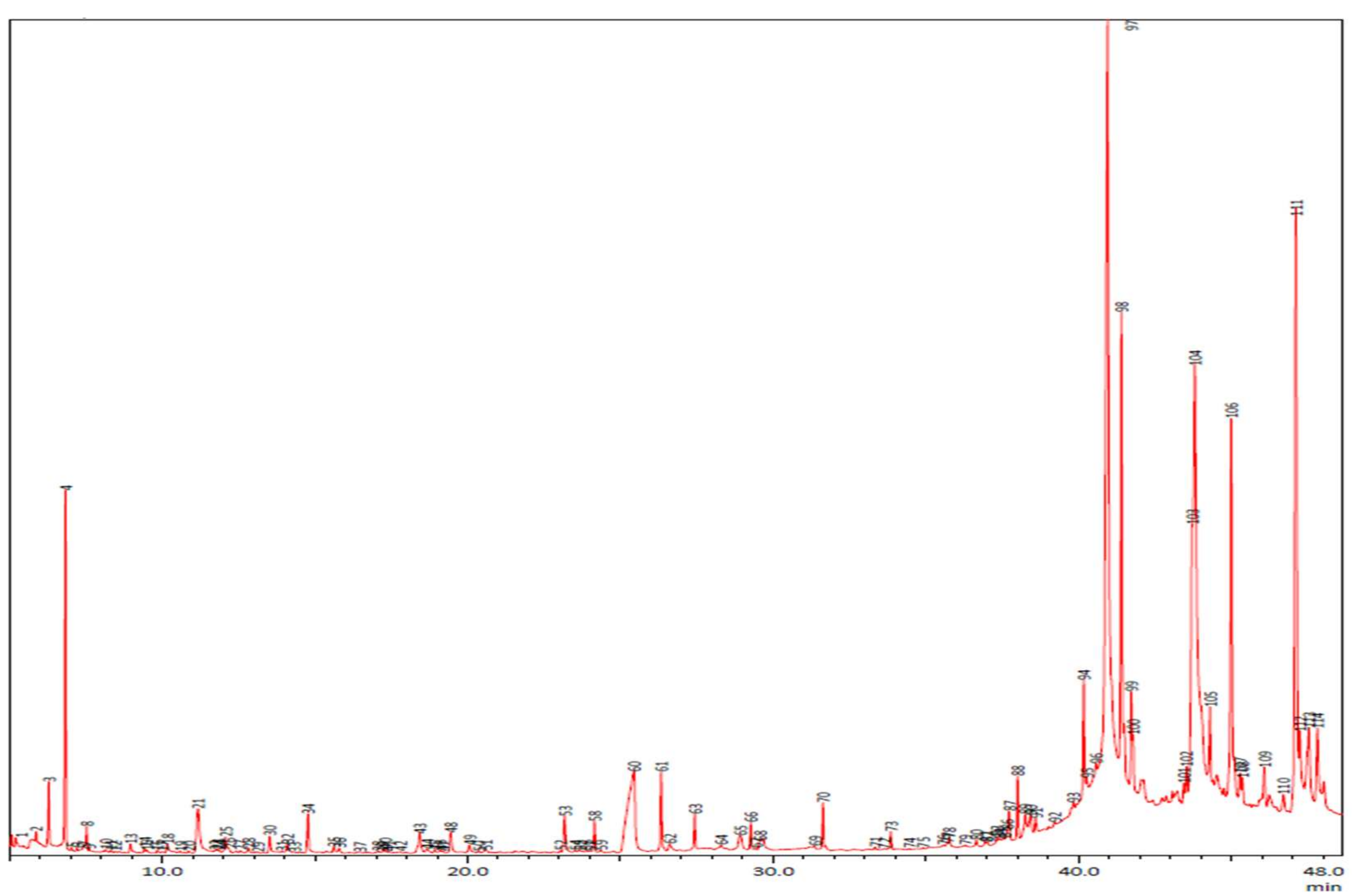

261 Fig 4. GC-MS chromatograph of Moringa *oleifera* seeds.

262

263 Table 2: Volatile compounds identified in Moringa *oleifera* seeds using GC-MS analysis

No.	Compounds	RT	Peak area (%)	No. on Chromatogram
	Esters			
1	Propanoic acid, 2-oxo-, methyl ester	6.29	0.76	3
2	Acetic acid, ethoxyhydroxy-, ethyl ester	7.04	0.04	5
3	Diethoxymethyl acetate	12.83	0.09	28
4	2-Propenoic acid, 2-methyl-, 2-hydroxypropyl ester	13.13	0.02	29
5	6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-	17.80	0.01	42
6	Cyclopentanecarboxylic acid, 4-tridecyl ester	19.18	0.03	47
7	1-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester	20.06	0.13	49
8	Hexanoic acid, 4-hexadecyl ester	28.29	0.07	64

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9	Phthalic acid, diethyl ester	29.57	0.14	68
10	2-Propenoic acid, pentadecyl ester	31.63	0.59	70
11	Acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester	33.63	0.02	72
12	Phthalic acid, dibutyl ester	35.69	0.07	77
13	Phthalic acid, diisobutyl ester	35.76	0.15	78
14	Palmitoleic acid, methyl ester	36.29	0.03	79
15	Pentadecanoic acid, 13-methyl-, methyl ester	36.64	0.09	80
16	9-Hexadecenoic acid, ethyl ester	37.65	0.15	86
17	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	37.99	0.78	88
18	Heptadecanoic acid, ethyl ester	38.42	0.46	90
19	Propanoic acid, 3-mercapto-, dodecyl ester	38.59	0.25	91
20	9-Octadecenoic acid, methyl ester, (E)- (Methyl elaidate)	40.16	1.52	94
21	Oleic acid, methyl ester (Methyl Oleate)	40.26	0.15	95
22	l-(+)-Ascorbic acid 2,6-dihexadecanoate	40.56	0.10	96
23	Ethyl Oleate	41.39	6.03	98
24	Octadecanoic acid, ethyl ester (Ethyl stearate)	41.77	0.53	100
25	9-octadecenyl ester (Oleyl oleate)	43.52	0.34	102
26	2,3-dihydroxypropyl elaidate	43.79	13.48	104
27	9-Octadecenoic acid, 1,2,3-propanetriyl ester	44.29	0.90	105
28	Docosanoic acid, ethyl ester	45.27	0.37	107
29	Oleoyl chloride	46.07	0.65	109
30	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	47.10	11.35	111
31	Glycidol stearate	47.52	1.76	113
32	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	47.80	1.57	114
	Alcohols			
33	1,4-Cyclohexanediol, trans-	7.22	0.02	6
34	1,2-Propanediol, 3-methoxy-	7.43	0.05	7
35	Ethanol, 2,2-diethoxy-	7.54	0.31	8
36	1,2,4-Butanetriol	9.50	0.04	15
37	Glycerin	11.18	1.35	21
38	1-Butanol, 4-(ethylthio)-	11.74	0.02	22
39	Methoxyacetaldehyde diethyl acetal	11.88	0.02	24
40	1-Dodecanol	18.43	0.48	43
41	1-Tetradecanol	24.15	0.42	58
42	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-	25.44	5.52	60
43	1-Tridecanol	26.33	1.06	61
44	3-Hexadecanol	31.35	0.05	69
45	3-Heptadecanol	35.56	0.06	76
	Aldehydes			
46	Butanal, 3-hydroxy-	8.38	0.00	12
47	Heptanal	10.01	0.02	17
48	Octanal	12.57	0.04	27
49	Nonanal	15.79	0.09	36
50	2-Methyl-oct-2-enedial	20.61	0.04	51
51	Undecanal	23.52	0.02	54

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52	Tridecanal	34.46	0.01	74
53	10-Octadecenal	37.42	0.09	84
54	9-Octadecenamide	37.55	0.09	85
55	cis-9-Hexadecenal	38.24	0.55	89
56	cis-13-Octadecenal	47.23	1.92	112
	Ketones			
57	2-Propanone, 1,1-dimethoxy-	6.84	3.86	4
58	2-Butanone	8.15	0.03	10
59	Dihydroxyacetone	8.97	0.14	13
60	1,2-Cyclopentanedione	10.17	0.14	18
61	2-Propanone, 1-(1,3-dioxolan-2-yl)-	10.87	0.04	20
62	1,3-Dioxol-2-one,4,5-dimethyl-	13.53	0.28	30
63	2-Heptanol, 5-ethyl-	14.31	0.01	33
64	2-Methyl-4-octanone	16.47	0.03	37
65	2-Pentanone, 3,4-epoxy-	18.93	0.03	45
66	1-Oxa-spiro[4.5]deca-6,9-diene-2,8-dione	37.01	0.05	82
67	Z-11-Pentadecenol	39.21	0.05	92
68	Cyclopentadecanone, 2-hydroxy-	39.82	0.13	93
69	Cyclopentadecanone	43.43	0.21	101
70	2-Tetradecanone	46.69	0.34	110
	Acids			
71	Acetic acid, (acetyloxy)-	5.88	0.18	2
72	Butanoic acid, 3-hydroxy-	10.59	0.03	19
73	Octanoic acid	17.42	0.03	41
74	Nonanoic acid	20.36	0.02	50
75	n-Hexadecanoic acid (Palmitic acid)	37.31	0.05	83
76	Oleic Acid	40.94	22.53	97
	Furans and lactones			
77	Furfural	7.62	0.02	9
78	2(5H)-Furanone	9.84	0.04	16
79	2-Hydroxy-gamma-butyrolactone	12.08	0.23	25
80	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	14.10	0.19	32
81	1,2-Ethanediol, 1-(2-furanyl)	19.10	0.03	46
82	5-Hydroxymethylfurfural	19.45	0.36	48
83	3-Deoxy-d-mannoic lactone	28.92	0.52	65
	Nitrogen containing compounds			
84	N,N-Dimethylaminoethanol	5.22	0.12	1
85	1,3,5-Triazine-2,4,6-triamine	14.78	0.68	34
86	Acetic acid, 2-(N-methyl-N-phosphonatomethyl)amino-	15.62	0.11	35
87	1-Heptadecanamine	18.67	0.07	44
88	Nonanamide	23.03	0.02	52
89	Dodecanamide (Lauryl amide)	33.33	0.04	71
90	Tetradecanamide (Myristic amide)	37.72	0.44	87
91	Hexadecanamide (Palmitic amide)	41.72	1.26	99
92	Docosenamide	44.99	6.04	106

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93	Nonadecanamide	45.35	0.32	108
	Sulfur containing compounds			
94	Sulfurous acid, cyclohexylmethyl hexadecyl ester	23.17	0.67	53
	Hydrocarbons			
95	1-Butene, 4,4-diethoxy-2-methyl-	9.43	0.07	14
96	1-Methyl-2-octylcyclopropane	12.17	0.07	26
97	2-Trifluoroacetoxytridecane	13.92	0.03	31
98	trans-2,3-Epoxynonane	23.64	0.03	55
99	2-Heptafluorobutyroxypentadecane	23.89	0.04	56
100	1,2-Epoxyundecane	24.02	0.02	57
101	Heptacosane	24.36	0.03	59
102	1-Heptadecene	29.26	0.33	66
103	Octadecane, 1,1'-[(1-methyl-1,2-ethanediyl)bis(oxy)]bis-	29.43	0.01	67
104	1-Nonadecene	33.85	0.22	73
	Pyrans			
105	Tetrahydro-4H-pyran-4-ol	17.07	0.03	38
106	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	17.20	0.02	39
	Others			
107	5,6-Dihydroxypiperazine-2,3-dione dioxime	8.32	0.02	11
108	Tetraethyl silicate	11.81	0.04	23
109	Silanediol, dimethyl-, diacetate	17.30	0.07	40
110	D-Allose	26.61	0.19	62
111	Phenol, 2,4-bis(1,1-dimethylethyl)-	27.43	0.51	63
112	Oxirane, hexadecyl-	34.90	0.02	75
113	Ethyl iso-allocholate	36.94	0.06	81
114	Oleic anhydride	43.70	3.96	103

264 [*] RT: Retention time in minutes.

265

266

267 3.3. Anti-proliferative activities of MOSs

268 The anti-proliferative effectiveness of the MOSs extracts were examined on the colon cancer cells
269 via the MTT assay. After 48 hrs of treatments, the cell viability status was examined in both normal (HEK-
270 293) and cancerous (HCT-116) cells. The percentage of cell viability and inhibitory concentration (IC_{50})
271 values of both normal and treated cells were calculated. The MTT assay results after 48 hrs of treatment
272 revealed the cytotoxic effects of the MOSs extracts, indicating their inhibitory action on the HCT-116
273 (cancerous) cells. The average of cell viability data showed (Fig 5) that the treatment using MOSs extracts

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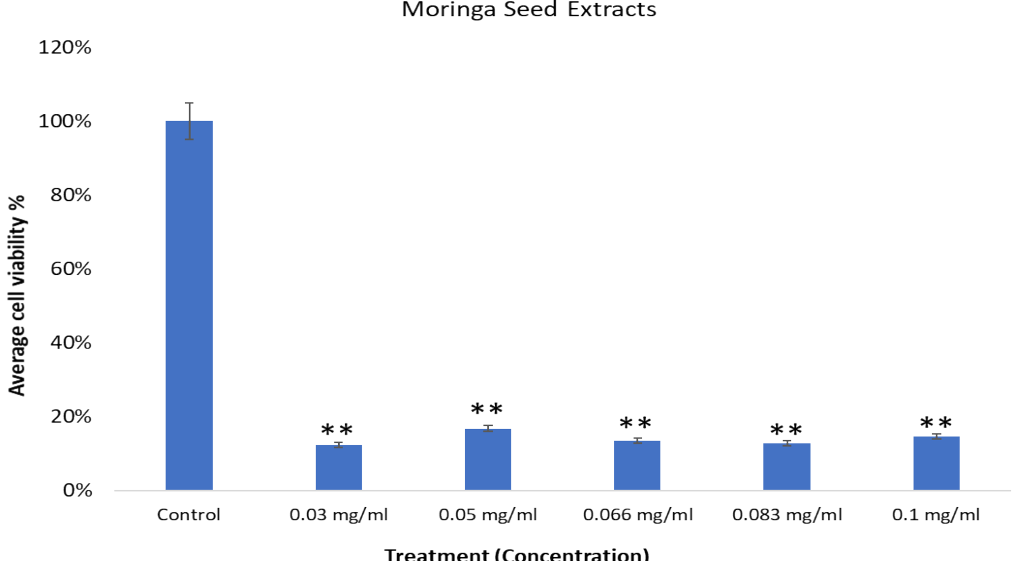
274 could induce a significant decrease in the cancer cells viability compared to the control cells (without
275 treatment using MOSs extracts).
276
277



278

279 **Fig 5.** Impact of *Moringa oleifera* seed extracts on the colon cancer cells. The HCT-116 treated with
280 moringa seed extracts for 48 hrs with different concentrations showing the average cell viability done by
281 MTT assay. (** $p < 0.01$)

282

283

284 The specificity of the MOSs extracts (at different concentrations ranged from 0.03 to 0.1 mg/ml)
285 on the normal and healthy (HEK-293) cells were inspected after 48 hrs of treatment using the MTT assays
286 (Fig 6). The results showed that the inhibitory activity of the MOSs extracts (irrespective of their
287 concentration) on the normal cells was insignificant, thereby suggesting the specificity of the MOSs
288 extract towards the colon cancer cells only.

289

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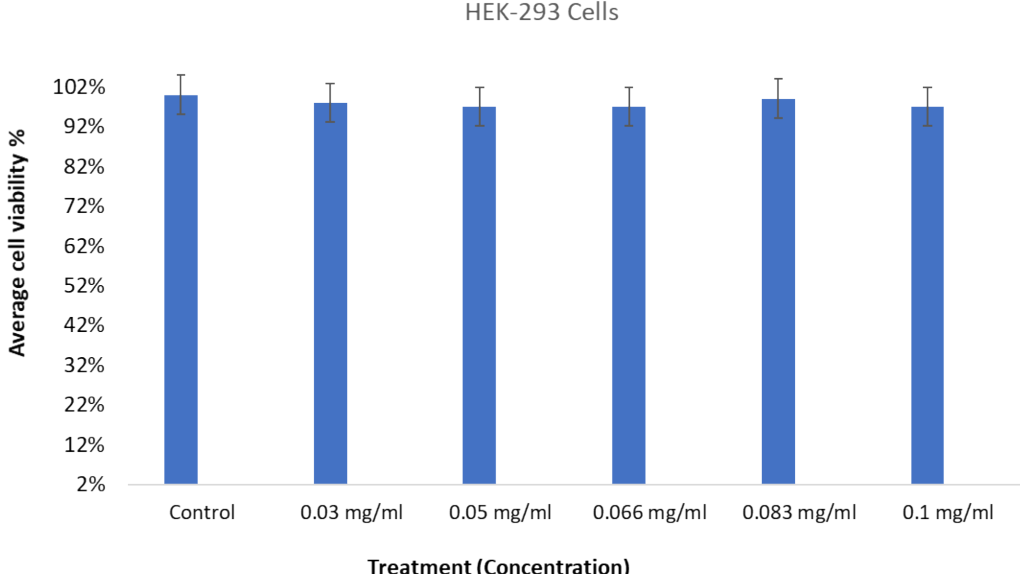

291

292 **Fig 6.** Impact of *Moringa oleifera* seed extracts on HEK-293 which were treated with moringa seed
293 extracts for 48h showing the average cell viability done by MTT assay. The cells were treated with
294 different concentrations (0.03 to 0.1 mg/ml) and. Data are the means ± SD of three different experiments.
295 Difference between two treatment groups were analysed by student's t test

296

297

298 3.4. Nuclear disintegration due to treatment of Moringa seed extracts

299 The morphologies of the untreated and MOSs extracts treated cells were imaged using the CSM as
300 shown in Fig 7. The cancerous cells (HCT-116) treated with MOSs extracts exhibited strong inhibitory
301 action (Fig 7(b)) than the control sample (Fig 7(a)).

302

303

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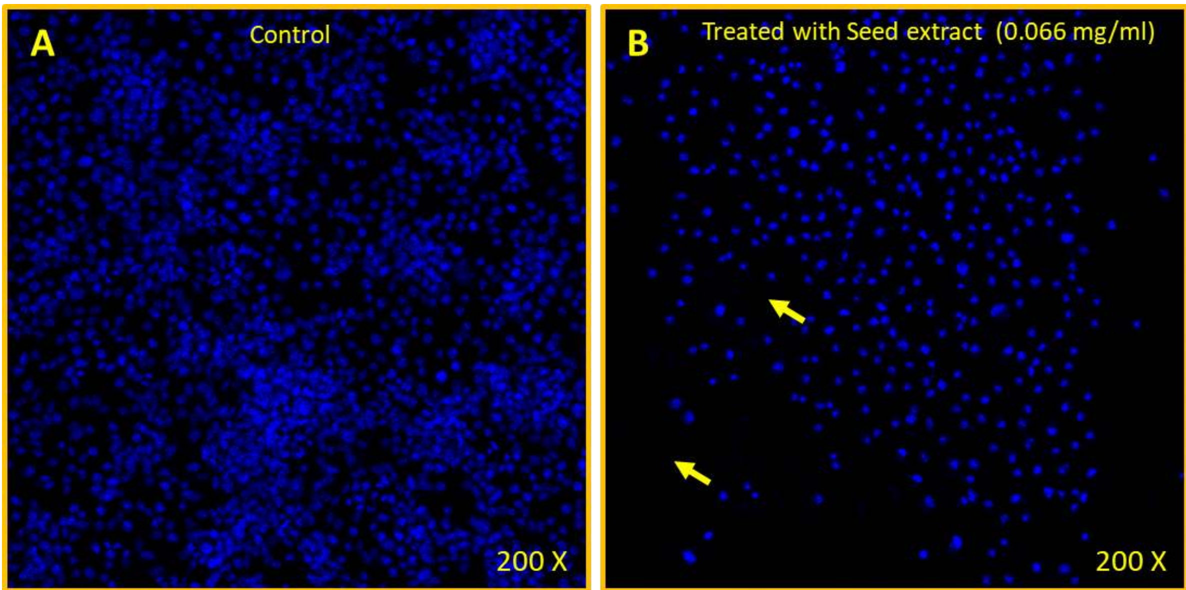

305

306 **Fig 7.** Nuclear staining by DAPI. The cancerous cells HCT-116 cells. (A) Control (nontreated) (B) treated
307 with seed extracts of *Moringa oleifera* (0.066 mg/mL). DAPI stained cells visualized through confocal
308 microscope and figure B show significant loss (arrows) of staining due to treatment. 200x magnifications.

309

310

311 It was inferred that the observed strong anti-cancerous activities of the MOSs extracts on the colon
312 cancer cells may be due to the presence of high level of oleic acid and fatty acid in the extracts as supported
313 by the GC-MS analysis. Yet, only few studies have been performed on the MOSs extracts indicating the
314 enhanced anti-cancerous potency of such extracts correlated to the occurrences of high oleic acid and fatty
315 acid contents [50-52].

316

317 3.5. Anti-microbial efficacy of MOSs extracts

318 The Agar well diffusion assay was used to determine the anti-microbial effectiveness of the
319 proposed MOSs extracts where the inhibited areas around the inoculated wells were measured. This zone
320 of inhibition was caused by the diffusion of the active chemical constituents of the MOSs extract around

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321 the inoculated wells. The results revealed the considerable impact of the MOSs extracts seed on the
322 bacterial strains (both gram-positive and gram-negative). However, the anti-bacterial action of the MOSs
323 extracts was better against the gram-positive *S. aureus* bacteria. Fig 8 shows the MOSs extracts
324 concentration dependent inhibition zones of the *S. aureus* bacteria (ranged from 18 to 24 mm). Conversely,
325 the inhibition zones of the *E. coli* were ranged from 6 to 20 mm wherein the maximum and minimum
326 inhibition zone diameter was obtained with the extracts concentration of 250 and 50 mg/ml, respectively
327 (Fig 9). The observed discrepancy in the bactericidal action of the MOSs extracts for two different types
328 of test bacteria may be due to the occurrence of their varying cell components [42].

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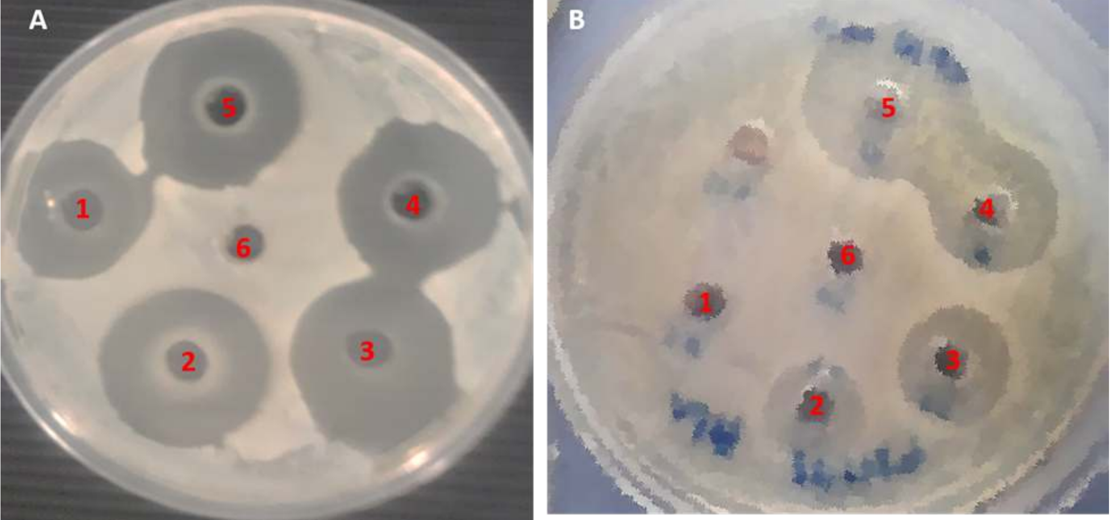

332

333 **Fig 8**. Agar well diffusion plates showing the zone of inhibition. (A) *S. aureus* (B) *E. coli.* **1**: 50 mg/ml,
334 **2**: 100 g/ml, **3**: 150 mg/ml, **4**: 200 mg/ml, **5**: 250 mg/ml of seed extract of *Moringa oleifera*.

335

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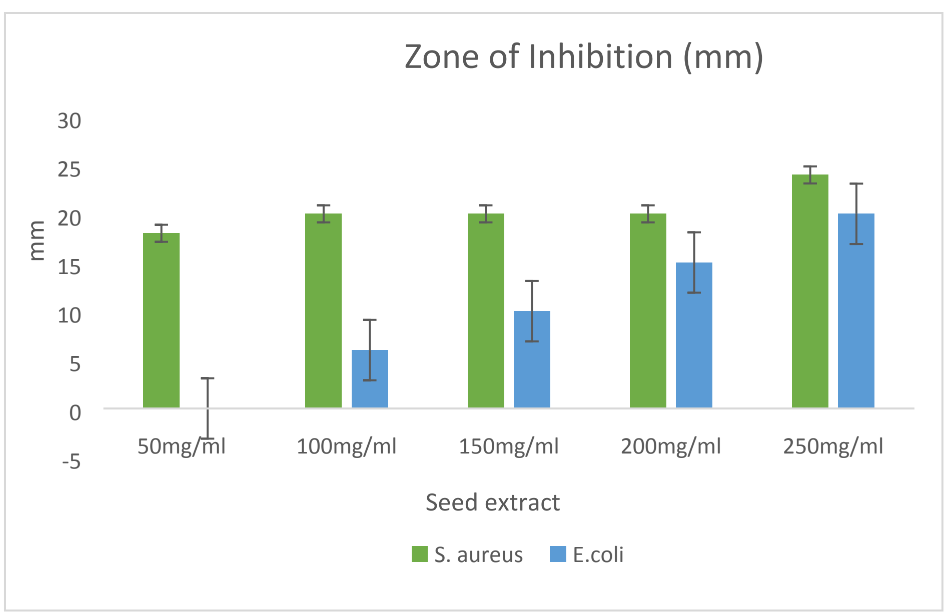

339

340 **Fig 9**. Zone of inhibition in millimeters (mm) against S. *aureus* and *E. coli* using the different
341 concentrations of *Moringa oleifera* seed extract.

342

343

344 ## 4. Conclusions

345 The LIBS and GC-MS technique was used to identify and quantify the elemental compositions of
346 the MOSs. The anti-cancerous and anti-microbial effectiveness of the MOSs extracts were evaluated for
347 the first time. The LIBS spectra revealed the presence of diverse elements in the MOSs useful for many
348 health benefits. The GC-MS analysis reconfirmed the presence of several anti-cancerous and anti-
349 microbial compounds in the MOSs extracts. The cell viability and DNA nuclear morphologies of the
350 HEK-293 and HCT-116 cells treated with the MOSs extracts were measured using the MTT assay and
351 DAPI staining, respectively. For the anti-cancer efficacy evaluation the HCT-116 were treated with MOSs
352 extracts for 48 hrs before the test. The viability of the HEK-293 cells was examined after treating those
353 using MOSs extracts. The cell viability percentage and IC_{50} values for both normal and cancerous cells
354 were assessed. The MTT assay showed a significant impact of the MOSs extract for inhibiting the growth
355 of the HCT-116 cells and insignificant inhibitory action of such extracts on the HEK-293 cells, indicating

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356 the excellent specificity of the extracts towards cancer cells. The MOSs extracts showed strong anti-
357 microbial activity in terms of the growth inhibition when tested with *S. aureus* and *E. coli* bacteria using
358 Agar well diffusion assay. It is established that MOSs extract can be a prospective anti-cancer and anti-
359 microbial agent for the functional biomedicinal drug formulation.
360

361 **Availability of data and material:** The data generated during the current study are available from the
362 corresponding author upon request.
363

364 **Author Declaration:** We wish to confirm that there are no known conflicts of interest associated with
365 this publication and there has been no significant financial support for this work that could have
366 influenced its outcome.
367

368 **Authors' Consent:** All authors have approved the final version of the manuscript.
369

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377

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