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Research Paper

Potential oil resources from underutilized seeds of *Sterculia foetida*, L. - quality assessment and chemical profiling with other edible vegetable oils based on fatty acid composition, oxidative stability, antioxidant activity and cytotoxicity

Short title: Potential oil resources from seeds of *Sterculia foetida*, L.

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1 **Abstract**

2 Vegetable oils are integral part in production of manufactured food both in domestic and
3 industrial scale. Vegetable oil market is increasing upward with a CAGR of 3.25% during the
4 forecast period (2019-2024) all around the world. Therefore, it is necessary to find alternative
5 sources of vegetable oil to fulfil the scarcity in the market. Seeds of *Sterculia foetida*, L.
6 yielded considerable amount of oil (58.7 g/100g) as compared to other vegetable oils (such as
7 sunflower, ground nut, mustard, soybean). Fatty acids composition of all the five tested oils
8 showed that total fatty acid as well as unsaturated fatty acid percentage is higher in *Sterculia*
9 seed oil. Proximate and mineral composition analysis suggested that *Sterculia* oil is a good
10 source of protein, lipids, macro and micronutrients. Lowest TOTOX value (2.67) and higher
11 iodine value (132-144) indicated its higher oxidative stability and presence of greater number
12 unsaturated bands in the fatty acid moieties which is also beneficial for human health. *Sterculia*
13 oil exhibited lower IC₅₀ values in DPPH (825.73 µg/ml), and NO (111.98 µg/ml) radical
14 scavenging assays. In case of ABTS radical scavenging activity, no significant differences
15 were observed in groundnut, mustard, *sterculia* and soybean except sunflower. *Sterculia* oil
16 did not exhibit any cytotoxic effect on both normal and cancerous cell lines even at
17 concentrations of 40µg/ml as evident from MTT assay. Thus, seed oil of *Sterculia foetida* may
18 be a cost-effective and viable source of safe nutritious edible oils to combat the present market
19 demand.

20 **Key words:** Edible vegetable oil; *Sterculia foetida*, L; Fatty acids; Oxidative stability;
21 Cytotoxic activity

22 **1. Introduction**

23 With a rising standard of living as well as industrial growth, demand for vegetable oils (VO)
24 is also soaring in global market. It is expected that in 2014, global vegetable oil market is

25 subjected to exceed 275 million metric tons due to the growing health consciousness of the
26 consumers. Palm oil mainly dominated the world vegetable oil market by more than one-third
27 of the total vegetable oil consumption and rest of the market occupied by soybean oil, canola
28 oil and sunflower seed oil (Mielke, 2018). Though palm oil represents one potential source to
29 meet this demand yet its consumption is associated with high risk of cardiovascular disease
30 (WHO, 2003; Ismail et al, 2018) and also it is eco-destructive. In Asia-Pacific regions, vegetable
31 oil market extended upward steadily with a CAGR of 5.4% over the analysis period due to the
32 factors such as population growth, simultaneous growth of food commodities, changing
33 dietary habits; rapid urbanization; improving living standards; increasing crop yields and oil
34 production and growing biofuel production in countries such as Indonesia, Malaysia, Thailand,
35 the Philippines, China and India. At present, there is a great demand of VO internationally,
36 but alternative sources are very few. Therefore, it is necessary to find alternative sources of
37 VO from underutilized oil resources to fulfil the global scarcity of VO applying non-agriculture
38 land (Shi et al, 2019).

39 *Sterculia foetida*, L. commonly known as bastard poon, java olive, hazel sterculia, wild
40 almond, is a large, straight, deciduous tree. It grows up to 40m in height and 3m in girth and
41 its branches are arranged in whorls and spreading horizontally. This plant species is widely
42 distributed in different geographical regions of India, south-east Asia and east coast of Africa.
43 The lifespan of this tree is more than 100 years. Plants are readily propagated through seeds
44 and need no special care (Staples and Herbst, 2005). It can be grown in non-cultivated or even
45 in waste lands with minimum water and nutrient supply. The plant grows very fast and
46 produces seeds within 2-3 years. The productivity of *S. foetida*, L. is reported to approx.
47 2000kg of seeds from one tree in one year.

48 This tree has immense potential for many therapeutic applications. Calcium content of *S.*
49 *foetida* leaves is about 2.66% and also sufficient amount of protein and phosphorus are found
50 in the leaves (Prakash & Kaviarasan, 2012). The leaves of this plant exhibit numerous
51 medicinal properties such as laxative, carminative, anti-inflammatory, antioxidant,
52 antimicrobial, cytotoxicity, anti-diabetic, anti-hyperlipidemic and insecticidal activities (Naik
53 et. al, 2004; Hussain et al, 2014; Mujumdar et al, 2000; Vital et al, 2010; Rani &
54 Rajasekharreddy, 2010; Suganya et al, 2017). Methanol extract of *S. foetida* seed possess
55 antioxidant activity (Galla, 2012). The de-oiled seed cake is rich in protein (28-89%) that can
56 be used as animal and fish food supplements (Oliveira et al, 2000; Shamsundar & Paramjyothi,
57 2010).

58 Braga et al (2015) isolated and purified a new lectin with antibacterial and hemolytic
59 activity from the seeds of *S. foetida*, L. Recently, a novel dye was extracted from the fruit shell
60 waste of *S. foetida* which was applied on mulberry silk fabric to produce aesthetic coloration
61 and wellness properties such as ultra-violet (UV) protection and antibacterial properties
62 (Teli and Pandit, 2018).

63 The aim of our present study was to validate the edibility of *Sterculia* seed oil as an
64 alternative safe vegetable oil. Therefore, in this study, in-depth characterization of fatty acid
65 composition of *Sterculia* seed oil and four other commonly available vegetable oils along with
66 their oxidative stability, radical scavenging activity, proximate and element content and
67 cytotoxicity have been performed to validate its safe use as edible oil.

68 **2. Materials and Methods**

69 **2.1. Collection of seed samples**

70 *S. foetida*, L. seeds were collected from the campus of Indian Statistical Institute (ISI),
71 Kolkata, India and nearby areas in the month of December to January from 2017 to 2018.
72 Other commonly available edible oil seeds, namely sunflower, groundnut, mustard and
73 soybean, were collected from the local market during the same time. Kolkata (22°33'N and
74 88°20'E) is located in the gangetic delta region of West Bengal and in the eastern part of India.
75 It has a tropical wet-and-dry climate with an annual mean temperature of 26.8 °C (80 °F) and
76 annual rainfall of 1,582 mm (62 in). This region has alluvial soil as it is near sea level, with the
77 average elevation being 17 feet ([Weather Atlas, Kolkata, India, 2017](#)).

78 **2.2. Extraction of oil**

79 100 g of each ground seed samples were soaked in 500 ml of hexane in 1000 ml
80 capacity extraction flasks ([Armah-Agyeman et al, 2016](#)). The mixture was then vortexed at
81 3000 rpm by Mechanical Stirrer (Model No. DC Stirrer NZ-1000s AC220V, EYELA) for 2 h and
82 then filtered through sintered disc funnel. The recovered collected extract was concentrated
83 in a rotary vacuum evaporator (EYELA, Model No. N1-NW) and oil was recovered in the
84 concentrating flask.

85 **2.3. Proximate Composition of the Seeds**

86 Proximate composition of seeds was examined using the standard official methods of the
87 Association of Official Analytical Chemists ([AOAC, 2006](#)).

88 **2.4. Mineral Content of Seed Flour**

89 Mineral content was determined by following the method of [Pinheiro et al. \(2010\)](#). Seed
90 flour sample of 5.0 g was incinerated in a furnace at 550°C and the residues were dissolved in
91 50mL of 0.5M HNO₃ solution. The concentrations of K, Ca, Na, Mg, Zn, Cu, Mn, Pb and Fe were

92 measured by Atomic Emission Spectroscopy (AES) 4200 MP-AES SYSTEMS manufactured by
93 AGILENT TECHNOLOGIES. A calibration curve was prepared by using standard metal solution.

94 **2.5. Fatty Acid Composition of Oils**

95 For determining the fatty acid composition of all the tested oils, fatty acid methyl ester
96 (FAME) of oils were prepared according to the AOCS Official Method 996.06 (2001) with minor
97 alterations described by [Symoniuk et al. \(2017\)](#).

98 FAME samples of all the five oils along with standard fatty acids were subjected to
99 MDLC analysis (Model No. WATERS Technologies, 2695, LC System with 2487 inert Mass
100 Selective Detector) for determination of the fatty acids compositions. MDLC analysis of the
101 samples was done at Indian Institute of Chemical Biology, Jadavpur, Kolkata-700032, W.B.
102 India. The gradient run was for 15 mins which was programmed as follows –First 0 min for
103 column A it was 100% and for B it was 0%, next 2 mins it was 100% for A and for B it was 0%,
104 next 2.5 mins it was 80% and 20% respectively for column A and B. For next 4 mins, 4.5 mins,
105 8 mins, 8.5 mins, 12 mins ,12.5 mins and 15 mins for column A and column B were 80% and
106 20%, 70 % and 30 % ,80% and 20% ,80% and 20%, 100% and 0% and 100% and 0% respectively.
107 Total run was performed for 15 mins.

108 **2.6. Antioxidant Activities**

109 Different mechanisms such as free radical scavenging, reduction capacity, and metals
110 chelation, are employed to evaluate the antioxidant potential of a substance or a complex
111 mixture. In our study DPPH, ABTS and NO radical scavenging capacity assay were performed
112 to detect the antioxidant activities of all the five tested oils namely sterculia, sunflower,
113 groundnut, mustard and soybean.

114 **2.6.1. DPPH radical scavenging assay**

115 The DPPH radical scavenging activities of all the five oils were assessed in-vitro using
116 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical as described by [Pavithra and Vadivukkarasi](#)
117 [\(2015\)](#).

118 **2.6.2. ABTS scavenging capacity assay**

119 ABTS radical scavenging activity of all the five oils were determined by ABTS radical
120 cation decolorization assay [\(Re et al, 1999\)](#).

121 **2.6.3. Nitric oxide scavenging capacity assay**

122 Nitric oxide radical scavenging activity was measured spectrophotometrically
123 according to the method described by [\(Jagetia et al 2004\)](#) with minor modification.

124 **2.7. Determination of TOTOX value**

125 TOTOX value i.e. overall oxidation state of oils is calculated by using the formula AV +
126 2PV. Better quality of the oil is determined by its lower TOTOX value [\(Maszewska et al, 2018\)](#).

127 Peroxide value (PV) was measured according to the AOCS Official Method 965.33
128 [\(AOAC, 1999\)](#), and the content of secondary oxidation products i.e. p-anisidine value (p-
129 AV) was determined by the AOCS Official Method Cd 18-90 [\(AOCS, 2005\)](#).

130 **2.8. Determination of Iodine value**

131 Iodine value of oils is directly proportional to the degree of unsaturation of the product which
132 indicates the oxidative stability of the oil and was determined following the method of [Soares](#)
133 [and Rocha \(2018\)](#).

134 **2.9. Cytotoxicity Assay**

135 Cytotoxicity of *Sterculia* seed oil was measured by MTT assay on normal cell lines
136 (MCF-10A and HB2 - breast non-cancerous cell lines) as well as cancerous cell lines (AU-565,
137 BT-4T4 - breast cancer cell lines). These cell lines (MCF-10A, HB2, AU-565 and BT-4T4) were

138 procured from ATCC and maintained in Dulbecco's Modified Eagle Medium with 5% fetal calf
139 serum and antibiotics in 37°C incubator. Cells were seeded in 24 well culture plates in DMEM
140 growth medium at a density of 2.5×10^4 cells/well and incubated overnight in 37°C at 5% CO₂.
141 After 18 h, cells were treated with different concentrations of *Sterculia* oil (0 – 160 µg/ml i.e.
142 six set of experiments starting from concentrations 10 µg/ml , 20 µg/ml, 40 µg/ml, 80 µg/ml,
143 160 µg/ml including control) dissolved in DMSO, where the final concentration of DMSO was
144 kept below 1%. Further, after 24 h cells were washed with 1× PBS and then incubated with
145 0.5 mg/ml of MTT solutions (in 1× PBS) for 2.5 h (Mosmann, 1983 and Stockert, et al, 2018).
146 The Formazan crystals formed within the cells were dissolved using 400 µl of DMSO and
147 absorbance of the solution was measured at 570 nm on a multi well plate reader (Biotech
148 Instruments, USA).

149 **2.10. Statistical Analysis**

150 Values obtained from biochemical tests and antioxidant assays were analysed using
151 independent sample T-test and the significance level was set at $p < 0.05$. All the experiments
152 were replicated three times, and the data was represented as mean values and standard
153 deviation of the same. SPSS Statistics 19 software for Windows had been used for all statistical
154 analyses (SPSS 2009).

155 **3. Results**

156 **3.1. Proximate Composition of Seeds**

157 Proximate composition values of all the five tested oils namely sterculia, sunflower,
158 groundnut, mustard and soybean are shown in Table 1. Seed kernel of *S. foetida* produces
159 58.7g of oil per 100g of seeds as compared to 47.5 g, 49.8 g, 31.4 g and 16.3 g from seeds of
160 sunflower, ground nut, mustard and soybean respectively. Highest lipid content was found in

161 sterculia (58.4 g/100g) followed by sunflower (51 g), groundnut (50 g), mustard (36 g) and
162 soybean (18.39g). Significant amount of protein was found in sterculia (38.43 g), groundnut
163 (38.61 g) and soybean (37.69 g) per 100g of seeds. Soybean seeds contain higher amount of
164 soluble sugar (16.24 g/100g). Sterculia seeds retain lowest amount of moisture (5.28 g/100g)
165 in comparison to other tested oils. In the case of above mentioned oils, the total proximate
166 compositions of the oilseeds did not differ significantly as the seeds of Sterculia contain the
167 highest amount of oil and most minerals compared to other oils shown in [Table-1](#).

168 **3.2. Mineral Composition of Seed Flour**

169 Among the heavy metals, Sterculia seeds contained lowest amount of copper (16.67
170 mg/kg), lead (12.6 mg/kg), manganese (10.6mg/kg), iron (38.67 mg/kg) but high amount of
171 Zinc (138.33 mg/kg) which is found to be higher in sterculia as compared to other tested
172 seeds. Highest amount of lead (48.67 mg/kg) and iron (146.33 mg/kg) were detected in
173 mustard seeds whereas highest amount of copper (52.67 mg/kg) and manganese (62.2
174 mg/kg) were recorded from groundnut seeds ([Table 1](#)). Among the alkaline earth metals only
175 sodium (350.67 mg/kg), magnesium (3436 mg/kg) and potassium (19858 mg/kg) is found in
176 highest quantity in the seeds of sterculia compared to other oils as shown in the ([Table 1](#)).
177 Only calcium (5218.33 mg/kg) is present in highest quantity in the seeds of mustard compared
178 to other oil seed.

179 **3.3. Analyses of fatty acid composition**

180 The MDLC analysis of FAME samples of all the five tested oils shows the fatty acid
181 composition as shown in ([Table 2](#)).

182 In the five oils, the 6 fatty acids presents are linolenic acids (C18:3), linoleic acids
183 (C18:2), palmitic acid (C16:0), myristic acid (C14:0), oleic acid (C18:1) and sterculic acid

184 (C19:1). Of these the linolenic and linoleic acids are the polyunsaturated fatty acids (PUFA),
185 palmitic and myristic acid are the saturated fatty acids (SFA) and oleic acid is the
186 monounsaturated fatty acids (MUFA) and only sterculic acid is the cyclopropenoid fatty acids
187 (CFA). In the five oils, the carbon number in the fatty acids varies from C₁₄ to C₁₉. Total SFA
188 was present higher in stercuria oil (1.29 mg/ml) and lowest in groundnut oil (0.08mg/ml) other
189 than that rest other oils i.e sunflower oil, mustard oil and soybean oil consists of 0.23mg/ml,
190 0.77mg/ml and 0.77 mg/ml respectively. Total MUFA was present higher in sunflower oil
191 (0.62mg/ml) and lowest in stercuria oil (0.016mg/ml) and mustard, soybean and groundnut
192 oil consists of 0.18mg/ml, 0.14mg/ml and 0.02 mg/ml respectively. Finally total PUFA were
193 present in higher quantity in stercuria oil (1.44 mg/ml) and lowest in sunflower oil (0.56
194 mg/ml). The remaining mustard, groundnut and soybean oil consists of (0.88mg/ml),
195 (1.25mg/ml) and (0.72 mg/ml) fatty acids respectively. Calculating the total fatty acids it
196 shows that stercuria oil consists of highest amount of fatty acids i.e 3.296 mg/ml and
197 groundnut oil consists the lowest 1.35 mg/ml.

198 **3.4. Determination of TOTOX Value**

199 Oxidation of oil over time is measured by peroxide value (PV), para-anisidine value (AV)
200 and TOTOX value as shown in [Table 3](#).

201 Highest para-anisidine (p-AV) value was revealed by groundnut oil (5.45) followed by
202 mustard oil (5.09) whereas lowest p-AV value was detected in sunflower oil (2.99). Stercuria
203 and soybean oil showed moderate p-AV value i.e. 3.73 and 3.77 respectively.

204 Peroxide value of all the five tested oils ranges from 0.018 – 0.025 meq./kg oil. Stercuria
205 oil showed lowest TOTOX value (2.67) with higher oxidative stability followed by sunflower

206 (3.04) and soybean (3.81) oil. Higher TOTOX values were revealed by groundnut (5.48) and
207 mustard oil (5.13).

208 **3.5. Antioxidant activities**

209 Low IC₅₀ value is inversely related to high antioxidant capacity of the extract (Rufino et al,
210 2009).

211 **3.5.1. DPPH Radical Scavenging Activity**

212 DPPH radical scavenging activity of all the five oils were expressed in IC₅₀ (µg/ml) with BHT
213 as standard as shown in Figure 1. Highest antioxidant activity with lowest IC₅₀ value (797.98
214 µg/ml) was exhibited by soybean oil followed by groundnut (815.19 µg/ml) and sterculia
215 (825.96 µg/ml) oil. Mustard and sunflower oil revealed least DPPH radical scavenging activity
216 with IC₅₀ value of 1858.89 µg/ml and 1557.63 µg/ml respectively. In sterculia, groundnut and
217 soybean oil, there is no significant differences whereas sunflower and mustard oil showed
218 significant differences in DPPH activity.

219 **3.5.2. ABTS Radical Scavenging Activity**

220 ABTS radical scavenging activity of all the five oils were represented in IC₅₀ (µg/ml) as
221 shown in Figure 1. Sunflower oil showed highest ABTS⁺ scavenging activity with significantly
222 low IC₅₀ of 19.39 µg/ml. Soybean oil showed least antioxidant activity with highest IC₅₀ of
223 237µg/ml, followed by sterculia (IC₅₀ of 220 µg/ml), mustard (IC₅₀ of 199 µg/ml) and ground
224 nut (IC₅₀ of 169 µg/ml). No significant differences were observed in ABTS activity of
225 groundnut, mustard, sterculia and soybean except sunflower.

226 **3.5.3. NO Radical Scavenging Activity**

227 NO radical scavenging activity of all the five oils expressed in IC₅₀ (µg/ml) is given in Figure 1.
228 Sterculia oil exhibited highest NO radical scavenging activity with low IC₅₀ value of 114.98

229 $\mu\text{g/ml}$, followed by mustard (121.97 $\mu\text{g/ml}$), groundnut (123.14 $\mu\text{g/ml}$), soybean (230.24
230 $\mu\text{g/ml}$) and sunflower (266.64 $\mu\text{g/ml}$) oils. NO radical scavenging activity did not show any
231 significant differences in all the five tested oils.

232 **3.6. Cytotoxicity Assay**

233 Figure 2 showed the result of cytotoxic activity of sterculia seed oil on both normal
234 (MCF-10A and HB-2) and cancerous cell lines (BT-474 and AU-565) including control with
235 DMSO on MCF-10A cell line. From MTT assay it was revealed that the sterculia oil did not
236 show any cytotoxicity on both normal and cancer cell lines. On cancerous cell lines namely
237 BT-474 and AU-565, sterculia oil at 10 $\mu\text{g/ml}$ concentration exhibited 92.91% and 90.07%
238 survivability of cells respectively, at 20 $\mu\text{g/ml}$ concentration 92.38% and 85.12%, at 40 $\mu\text{g/ml}$
239 concentration 89.47% and 80.64% and at 80 $\mu\text{g/ml}$ concentration 82.46% and 74.01%
240 survivability of cells were noticed. On normal cell lines namely MCF-10A and HB-2, sterculia
241 oil at 10 $\mu\text{g/ml}$ concentration showed 94.40% and 92.08% survivability of cells respectively
242 whereas at 20 $\mu\text{g/ml}$ concentration 92.62% and 85.35%, at 40 $\mu\text{g/ml}$ concentration 86.61 and
243 84.42% and at 80 $\mu\text{g/ml}$ concentration 80.56% and 76.89% survivability of cells were
244 detected. The control experiment was done with DMSO on all cell lines in the same
245 concentrations as the tested samples. Minor reduction in survivability of cells was observed
246 due to the presence of DMSO as the solvent.

247 **4. Discussion**

248 Vegetable oil market in the world is expanding upward with a CAGR of 3.25% during
249 forecast period from 2019 to 2024. Domestic consumption of edible oils in Asian Pacific
250 countries like India, China, Indonesia, and Malaysia has increased substantially over the years
251 due to upliftment of economic condition, urbanization, changing dietary habits and proclivity

252 of processed foods. The country's vegetable oil consumption was at 23 million tonnes in 2017
253 and it will be expanded by three per cent annually to exceed 34 million tonnes by 2030
254 according to the Rabo Research Report (PTI, Mumbai, June 25, 2018). Because of stagnant
255 domestic vegetable oil supplies, over increasing demand will be filled by extending import
256 volumes. Thus, it is high time to explore alternative rich natural source of vegetable oil and
257 we show here that *Sterculia* seed oil could provide a viable source of nutritious edible oil.

258 Seeds of *S. foetida* yielded substantial amounts of oil (58.7 g per 100 g seed). The
259 moisture content of *Sterculia* seeds was 5.28%, which is low as compared to other tested oils.
260 Due to low moisture content, the seeds of *S. foetida* may have a prolonged shelf life. The
261 seeds contained significant amounts of crude oil, protein, lipid and minerals that include
262 heavy metals and alkaline earth metals. Heavy metals were present in very low amount
263 except zinc which is essential for proper functioning of the immune system. Alkaline earth
264 metals that are present includes magnesium, sodium and potassium present in higher
265 quantity in *sterculia* oil compared to other oilseeds except calcium that is present in lower
266 quantity. These earth metals potassium and sodium are electrolytes needed for the body to
267 function normally and help maintain fluid and blood volume in the body, and magnesium is
268 necessary for the formation of bone and teeth and for normal nerve and muscle function.

269 Fatty acids like palmitic acid, linoleic acid, linolenic acids, stearic acid and oleic acids
270 were common in all the five oils. Results reveal that *sterculia* oil consists of highest amount
271 of fatty acids, which includes the SFA, MUFA and PUFA. Highest percentage of
272 polyunsaturated fatty acids such as linoleic acids and linolenic acids and monounsaturated
273 fatty acids such as oleic acids along with *sterculic* acid (CFA) are present in *sterculia* oil that
274 helps in increasing the high density lipoprotein (HDL) i.e the good cholesterol which assists in
275 the removal of triacyl glycerols from the bloodstream (J.Lunn, & Theobald, 2006).

276 Unsaturated fats helps to reduce the risk of heart disease and lower the cholesterol as they
277 replace saturated fats in the diet [EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)
278 2010]. Along with this the data also shows that PUFA are present in higher quantity than the
279 MUFA in the sterculia oil. This is because the PUFA has more than one double bond in their
280 structure than MUFA that help lower blood cholesterol and also contain omega-3 and omega-
281 6 fatty acids that the body needs for proper brain function and cell growth. Omega-3 fats lower
282 trygliceride levels and increases the HDL (good cholesterol) levels (Schriber, A. Medline Plus
283 NIH US National Library of Medicine). Moreover, the unique cyclopropenoid fatty acid i.e.
284 sterculic acid [namely 8-(2-Octacyclopropen-1-yl) octanoic acid] was found in the Sterculia
285 seed oil (Kale et al, 2011; Vipunungeun and Chanida, 2009). Sterculic acid is a potent natural
286 product to fight against obesity by suppressing a bodily enzyme associated with insulin
287 resistance, which could indirectly help with reducing belly fat (Bao et al, 2003). It is also known
288 to inhibit of SCD1 (Stearoyl-CoA desaturase-1), a major enzyme involved in the control of lipid
289 metabolism and has emerged as a potential therapeutic target for reducing obesity and its
290 associated metabolic complications including insulin resistance and hepatic steatosis
291 (Ortinou, et al, 2013). This sterculic acid directly inhibits SCD activity, possibly by a turnover-
292 dependent reaction, without affecting the processes required for adipocyte
293 differentiation, *scd* gene expression or SCD protein translation (Gomez et al, 2003). So
294 Sterculia oil has a promise to act to reduce some factors causing obesity.

295 Lowest TOTOX value and higher iodine value of Sterculic oil indicates its higher
296 oxidative stability and presence of greater number of double bonds in the fatty acid moieties
297 which further supports that this oil would be beneficial for edible purposes.

298 No significant differences in IC₅₀ values among the oils were observed as measured by
299 NO radical scavenging activity. ABTS radical scavenging activity showed significant difference

300 only with sunflower oil whereas DPPH showed differences with sunflower and mustard oil. So
301 Sterculia oil is comparable to other vegetable oil based on their radical scavenging activity.

302 Furthermore, sterculia oil did not reveal any cytotoxic effect even at 40µg/ml
303 concentration against normal (MCF-10A and HB-2) and cancerous cell lines (BT-474 and AU-
304 565). So, it can be suggested that this oil has no toxicity on human beings and safe for human
305 consumption, however, further studies are to be performed in order to ensure the same. All
306 the parameters of sterculia oil recommends that the seed oil of *S. foetida*, L. may be an
307 alternative source of safe edible oil. As an additional fact, the seeds of *Sterculia apetala* are
308 reported to be commonly used in some tropical areas in Mexico for human and animal
309 nutrition (Herrera-Meza et al, 2014). Consumption of *S. apetala* seed oil in Zucker rats reduces
310 anxiety-like behaviour and some behavioural alterations in locomotor activity tests (Herrera-
311 Meza et al, 2017).

312 Because of the need of edible oil in the global market, seeds of this tree would be a
313 viable resource of nutritious, non-toxic edible vegetable oil. This plant has a wide range of
314 distribution in all around the world so it is not invasive nor eco-destructive. It can be cultivated
315 in un-utilized lands or even in waste lands with very nominal water and nutrient supply which
316 is very relevant to developing countries. Moreover, this tree can be used for backyard
317 planting, coastal protection and stabilization, urban greening, shade tree, large road side tree,
318 wild grafting (Orwa et al, 2009).

319 **5. Conclusions**

320 From our study it was revealed that *Sterculia foetida* seeds yield considerable
321 amount of oil (58.7 g/ 100 g) among all the tested vegetable oil seed samples. Proximate
322 and mineral composition analysis suggests that these seeds are rich and could be considered

323 as an alternative source of oil, protein, and micronutrients. Fatty acid composition of all the
324 tested oils showed that seed oil of sterculia contains highest amount of total fatty acids that
325 includes both PUFA and SFA and also CFA. So, it might be substituted with other edible
326 vegetable oils. This oil has a very low TOTOX value and high iodine value as compared to
327 other vegetable oils which indicates its higher oxidative stability and higher degree of
328 unsaturation which might be beneficial for our health. Radical scavenging activity
329 supported the similar nature of sterculia oil to the other edible oils. This oil does not have
330 any toxicity on human health as revealed from MTT assay on both normal and cancerous
331 cell lines. Based on all the tested parameters, we may conclude that this oil may serve as
332 an alternative, viable source of safe edible oil.

333 **Conflicts of Interest**

334 The authors declare that they have no conflicts of interest regarding publication of
335 this paper.

336 **Authorship Contribution Statement**

337 Ekta Bhattacharya & Rahul Bose – Investigation, collection of test data, formal
338 analysis, drafting the article; Suparna Mandal Biswas – Writing original draft, funding
339 acquisition, project administration, validation, supervision. Thomas Hughes and Arindam
340 Pramanik – Design of the experiment, investigation, formal analysis and made the critical
341 revision of the article.

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482

483

484 **Figure and Captions**

485 **Figure 1.** Antioxidant activities of all the five oils (namely Sterculia, Sunflower, Groundnut,
486 Mustard and Soybean) expressed in IC₅₀ (µg/ml). All the data are mean ± SD of triplicate
487 measurements. Bars with '*' are significantly different. IC₅₀, inhibitory concentration;
488 DPPH, DPPH radical scavenging activity; ABTS, ABTS radical scavenging activity; NO,
489 nitrite scavenging activity.

490 **Figure 2.** MTT assay of Sterculia seed oil at different concentrations on normal (MCF-10A and
491 HB2) and cancer (AU-565, BT-4T4) cell lines.

492 **Table 1.** Proximate and mineral compositions of *Sterculia foetida*, *L.* seeds along with
493 sunflower, groundnut, mustard and soybean seeds.

494 **Table 2.** Comparative fatty acids profiling of all the five seed oils namely sterculia, sunflower,
495 groundnut, mustard and soybean based on GCMS analyses of the FAME samples of
496 respective oils.

497 **Table 3.** Iodine value and oxidation status of five seed oils namely Sterculia, Sunflower,
498 Groundnut, Soybean and Mustard.

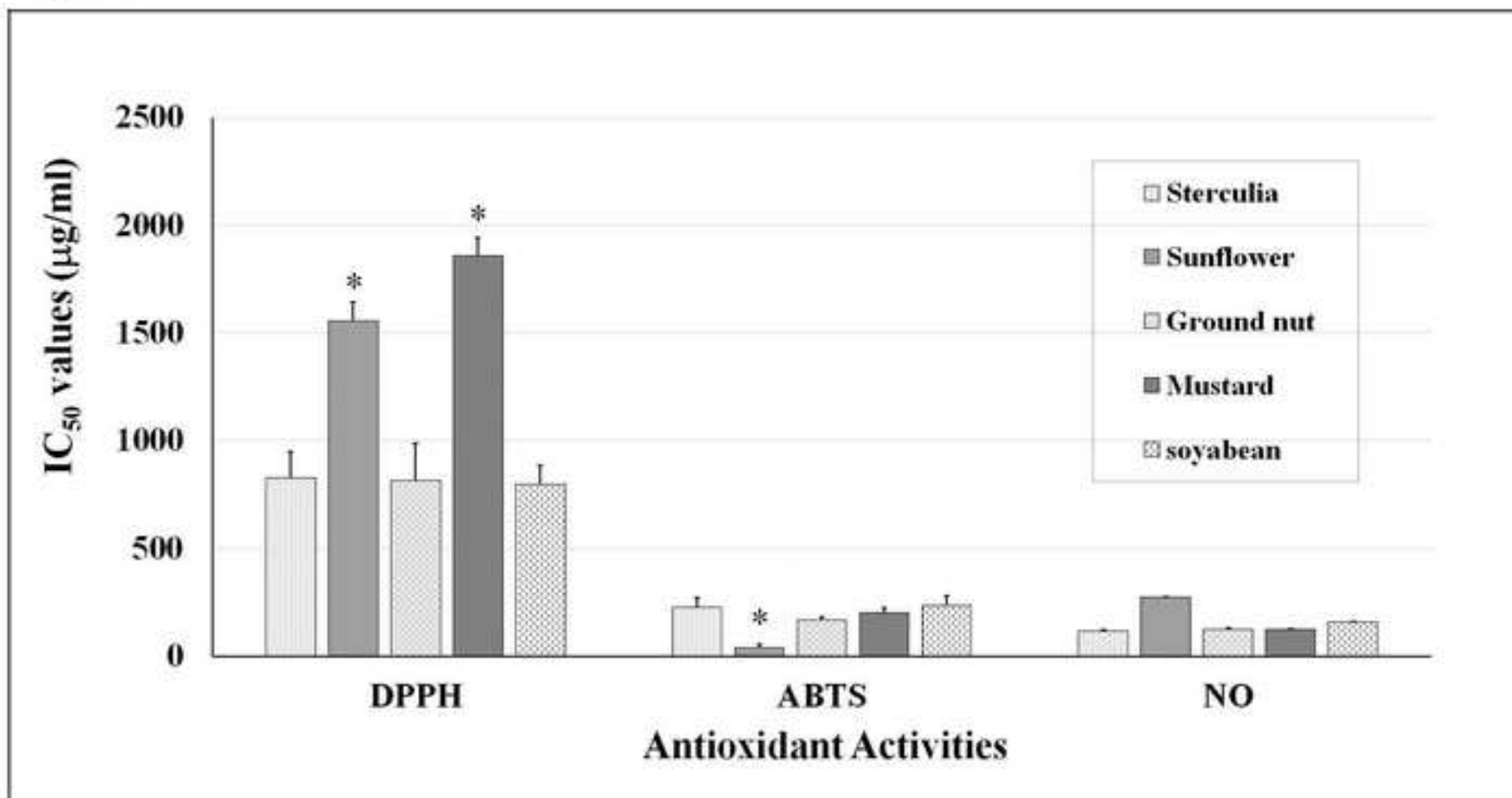
Figure 1

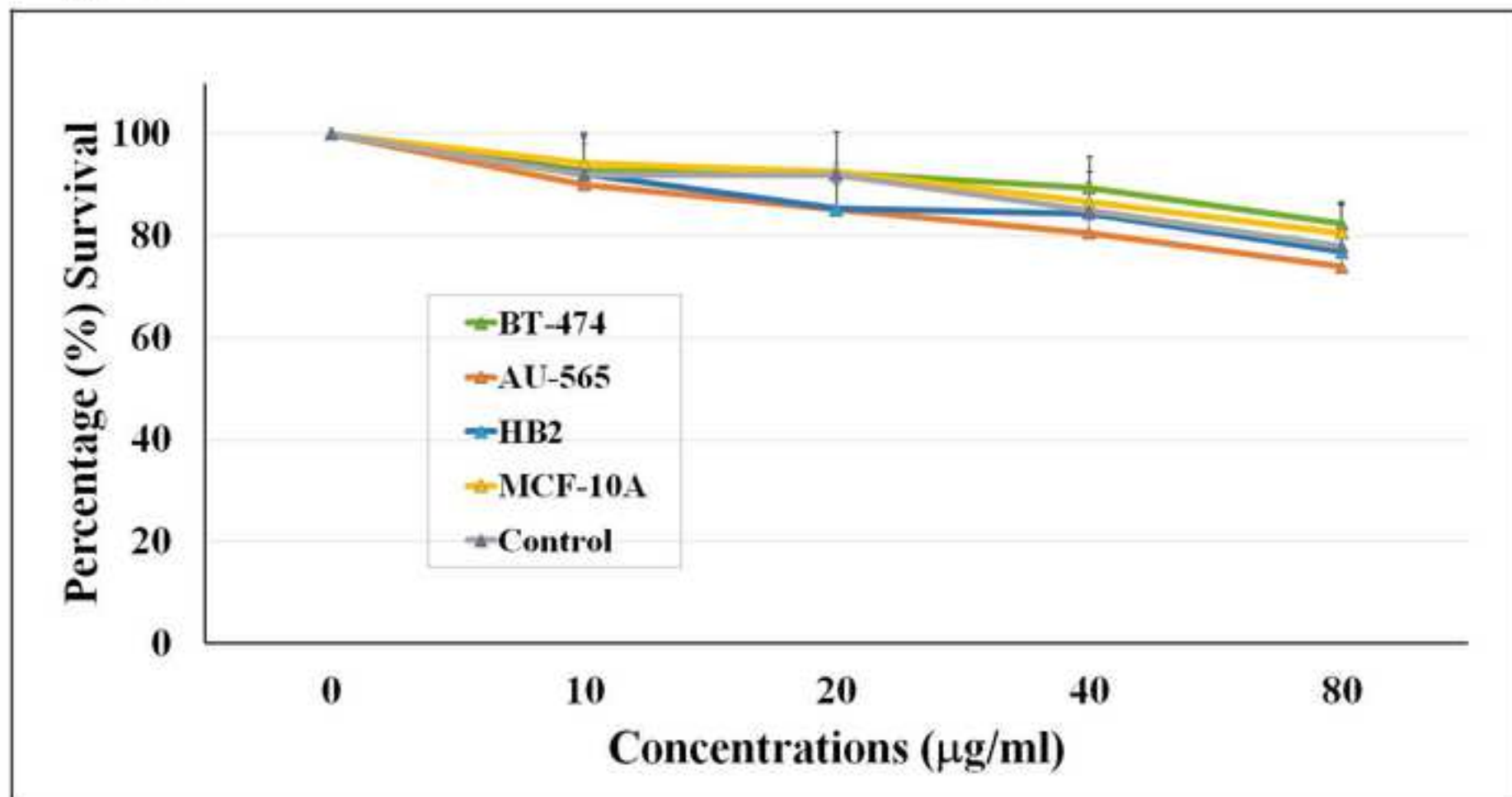
Figure 2

Table 1. Proximate and mineral compositions of *Sterculia foetida* seeds along with Sunflower, Groundnut, Mustard and Soyabean seeds.

	Sterculia	Sunflower	Groundnut	Mustard	Soybean
NUTRITIONAL COMPONENTS (g/100g)					
Oil Content	58.7± 2.37^a	47.5± 2.22^a	49.8± 1.62^a	31.4± 1.45^b	16.3± 1.19^c
Lipid Content	58.4± 4.52^a	51± 3.50^a	50± 6.52^a	36± 3.36^a	18.39± 4.52^b
Protein Content	38.43± 2.81^a	20.91± 3.08^c	38.61± 3.02^a	28.8± 4.05^b	37.69± 2.66^a
Soluble Sugar Content	1.73±1.34^c	2.54± 1.22^c	1.46± 1.26^c	6.74±1.05^b	16.24±2.73^a
Moisture Content	5.28± 3.22^b	7.18± 3.22^b	5.98± 2.01^b	9.12± 4.33^a	13.5± 2.44^a
ALKALINE EARTH METALS (mg/kg)					
Magnesium	3436 ±4.51^b	3251±3.61^a	590.33 ±6.55^a	3040±72.11^a	2756.67±40.5^a
Sodium	350.67±24.44^a	89.33±3.05^b	335±4.35^a	52.67±6.42^b	181±3.60^a
Potassium	19858±345.5^b	6455.67±6.0^b	14500±2.01^a	6882±65.21^b	5293.33±15.6^a
Calcium	590.33±4.51^c	3251±3.61^a	2002.33±5.8^b	5218.33±7.6^a	3124±5.29^a
HEAVY METALS (mg/kg)					
Copper	16.67± 4.16^b	20±2.03^b	52.67±2.08^a	38.67±1.15^a	48±3.0^a
Lead	12.6± 3.05^b	10.33±1.52^b	44±2.35^a	48.67± 8.14^a	38.33±5.50^a
Manganese	10.6± 1.15^b	21±2.01^b	62±2.64^a	41.33±2.5^a	41±1.03^a
Iron	38.67± 2.51^b	53.33±1.52^b	111±2.64^a	146.33±1.52^a	38.67±2.51^b
Zinc	138.33±2.08^a	52.67±3.05^b	75±5.56^a	38.67±1.15^b	52.67±1.52^b

Mean values ± standard deviation for $n = 3$

*Values (means± SD) with different index letters are statistically significantly different ($P < 0.05$).

Table 2. Fatty acid composition of all the five seed oils namely Sterculia, Sunflower, Groundnut, Mustard and Soybean based on MDLC analyses of the FAME samples of respective oils.

Name of the fatty acid	Fatty acid content (mg/ml)				
	Sterculia	Sunflower	Mustard	Groundnut	Soyabean
Sterculic acid (C 19:1) CFA	0.55± 0.01	0	0	0	0
Linolenic acid (C 18:3) PUFA	1.12± 0.01	0.52±0.01	0.76±0.00	1.25±0.05	0.62± 0.02
Linoleic acid (C18:2) PUFA	0.32±0.040	0.04±0.003	0.12±0.039	0	0.1± 0.02
Palmitic acid (C16:0) SFA	1.23± 0.16	0.20± 0.04	0.76±0.032	0.04±0.002	0.76± 0.029
Myristic acid (C14:0) SFA	0.06± 0.001	0.033± 0.001	0.015±0.002	0.04± 0.001	0.012± 0.009
Oleic Acid (C18:1) MUFA	0.016± 0.006	0.62± 0.13	0.18± 0.019	0.024± 0.011	0.142± 0.00
Total saturated fatty acids	1.29± 0.015	0.233± 0.025	0.775± 0.017	0.08± 0.012	0.772± 0.019
Total monounsaturated fatty acids	0.016± 0.006	0.62± 0.013	0.18± 0.019	0.024± 0.011	0.142± 0.00
Total polyunsaturated fatty acids	1.44± 0.020	0.56± 0.016	0.88± 0.019	1.25± 0.005	0.72± 0.015
Total cyclopropenoid fatty acids	0.55± 0.001	0	0	0	0
TOTAL	3.296± 0.013	1.413± 0.019	1.835± 0.018	1.354± 0.072	1.634± 0.023

Table 3. Iodine value and oxidation status of five seed oils namely Sterculia, Sunflower, Ground nut, Soyabean, Mustard.

SEED OIL	Para-Anisidine Value (p-AV)	Peroxide value (PV) (meq./kg oil)	TOTOX (2 PV + p-AV)	Iodine Value
Sterculia	3.73 ± 0.64	0.023 ± 0.0034	2.67 ± 0.72	132-144
Sunflower	2.99 ± 0.45	0.025 ± 0.0052	3.04 ± 0.46	122-140
Ground nut	5.45 ± 0.68	0.018 ± 0.0004	5.48 ± 0.68	87-106
Mustard	5.09 ± 0.87	0.021 ± 0.005	5.13 ± 0.88	94-111
Soyabean	3.77 ± 0.90	0.020 ± 0.0004	3.81 ± 0.90	120-134

The values are mean ± SD of three independent experiments

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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