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

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

#### 22 1. Introduction

With a rising standard of living as well as industrial growth, demand for vegetable oils (VO)
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 consumers. Palm oil mainly dominated the world vegetable oil market by more than one-third 26 of the total vegetable oil consumption and rest of the market occupied by soybean oil, canola 27 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 (WHO, 2003; Ismail et al, 2018) and also it is eco-destructive. In Asia-Pacific regions, vegetable 30 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 dietary habits; rapid urbanization; improving living standards; increasing crop yields and oil 33 34 production and growing biofuel production in countries such as Indonesia, Malaysia, Thailand, the Philippines, China and India. At present, there is a great demand of VO internationally, 35 but alternative sources are very few. Therefore, it is necessary to find alternative sources of 36 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 its branches are arranged in whorls and spreading horizontally. This plant species is widely 41 distributed in different geographical regions of India, south-east Asia and east coast of Africa. 42 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 in waste lands with minimum water and nutrient supply. The plant grows very fast and 45 produces seeds within 2-3 years. The productivity of *S. foetida*, L. is reported to approx. 46 47 2000kg of seeds from one tree in one year.

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

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

The aim of our present study was to validate the edibility of *Sterculia* seed oil as an alternative safe vegetable oil. Therefore, in this study, in-depth characterization of fatty acid composition of *Sterculia* seed oil and four other commonly available vegetable oils along with their oxidative stability, radical scavenging activity, proximate and element content and 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), Kolkata, India and nearby areas in the month of December to January from 2017 to 2018. 71 Other commonly available edible oil seeds, namely sunflower, groundnut, mustard and 72 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 capacity extraction flasks (Armah-Agyeman et al, 2016). The mixture was then vortexed at 80 3000 rpm by Mechanical Stirrer (Model No. DC Stirrer NZ-1000s AC220V, EYELA) for 2 h and 81 82 then filtered through sintered disc funnel. The recovered collected extract was concentrated in a rotary vacuum evaporator (EYELA, Model No. N1-NW) and oil was recovered in the 83 84 concentrating flask.

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

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

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

89 Mineral content was determined by following the method of Pinheiro et al. (2010). Seed flour sample of 5.0 g was incinerated in a furnace at 550°C and the residues were dissolved in 90 50mL of 0.5M HNO<sub>3</sub> solution. The concentrations of K, Ca, Na, Mg, Zn, Cu, Mn, Pb and Fe were 91

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

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

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

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

#### 108 2.6. Antioxidant Activities

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

- ABTS radical scavenging activity of all the five oils were determined by ABTS radical
  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

TOTOX value i.e. overall oxidation state of oils is calculated by using the formula AV +
2PV. Better quality of the oil is determined by its lower TOTOX value (Maszewska et al, 2018).
Peroxide value (PV) was measured according to the AOCS Official Method 965.33
(AOAC, 1999), and the content of secondary oxidation products i.e. p-anisidine value (pAV) 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

procured from ATCC and maintained in Dulbecco's Modified Eagle Medium with 5% fetal calf 138 serum and antibiotics in 37°C incubator. Cells were seeded in 24 well culture plates in DMEM 139 growth medium at a density of  $2.5 \times 10^4$  cells/well and incubated overnight in 37°C at 5% CO<sub>2</sub>. 140 After 18 h, cells were treated with different concentrations of *Sterculia* oil  $(0 - 160 \mu g/ml i.e.$ 141 six set of experiments starting from concentrations 10 μg/ml, 20 μg/ml, 40 μg/ml, 80 μg/ml, 142 160 μg/ml including control) dissolved in DMSO, where the final concentration of DMSO was 143 144 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  $\mu$ l of DMSO and 147 absorbance of the solution was measured at 570 nm on a multi well plate reader (Biotech Instruments, USA). 148

#### 149 **2.10. Statistical Analysis**

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

### 155 **3. Results**

#### 156 **3.1. Proximate Composition of Seeds**

Proximate composition values of all the five tested oils namely sterculia, sunflower, groundnut, mustard and soybean are shown in Table 1. Seed kernel of *S. foetida* produces 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 sunflower, ground nut, mustard and soybean respectively. Highest lipid content was found in sterculia (58.4 g/100g) followed by sunflower (51 g), groundnut (50 g), mustard (36 g) and soybean (18.39g). Significant amount of protein was found in sterculia (38.43 g), groundnut (38.61 g) and soybean (37.69 g) per 100g of seeds. Soybean seeds contain higher amount of soluble sugar (16.24 g/100g). Sterculia seeds retain lowest amount of moisture (5.28 g/100g) in comparison to other tested oils. In the case of above mentioned oils, the total proximate compositions of the oilseeds did not differ significantly as the seeds of Sterculia contain the highest amount of oil and most minerals compared to other oils shown in Table-1.

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

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

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

180 The MDLC analysis of FAME samples of all the five tested oils shows the fatty acid181 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

(C19:1). Of these the linolenic and linoleic acids are the polyunsaturated fatty acids (PUFA), 184 palmitic and myristic acid are the saturated fatty acids (SFA) and oleic acid is the 185 monounsaturated fatty acids (MUFA) and only sterculic acid is the cyclopropenoid fatty acids 186 (CFA). In the five oils, the carbon number in the fatty acids varies from C<sub>14</sub> to C<sub>19</sub>. Total SFA 187 188 was present higher in sterculia 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, 0.77mg/ml and 0.77 mg/ml respectively. Total MUFA was present higher in sunflower oil 190 191 (0.62mg/ml) and lowest in sterculia oil (0.016mg/ml) and mustard, soybean and groundnut oil consists of 0.18mg/ml, 0.14mg/ml and 0.02 mg/ml respectively. Finally total PUFA were 192 present in higher quantity in sterculia oil (1.44 mg/ml) and lowest in sunflower oil (0.56 193 194 mg/ml). The remaining mustard, groundnut and soybean oil consists of (0.88mg/ml), (1.25mg/ml) and (0.72 mg/ml) fatty acids respectively. Calculating the total fatty acids it 195 196 shows that sterculia 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** 

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

Highest para-anisidine (p-AV) value was revealed by groundnut oil (5.45) followed by mustard oil (5.09) whereas lowest p-AV value was detected in sunflower oil (2.99). Sterculia 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. Sterculia
 205 oil showed lowest TOTOX value (2.67) with higher oxidative stability followed by sunflower

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

#### 208 **3.5.** Antioxidant activities

Low IC<sub>50</sub> value is inversely related to high antioxidant capacity of the extract (Rufino et al,
2009).

#### 211 **3.5.1. DPPH Radical Scavenging Activity**

DPPH radical scavenging activity of all the five oils were expressed in IC<sub>50</sub> ( $\mu$ g/ml) with BHT as standard as shown in Figure 1. Highest antioxidant activity with lowest IC<sub>50</sub> value (797.98  $\mu$ g/ml) was exhibited by soybean oil followed by groundnut (815.19  $\mu$ g/ml) and sterculia (825.96  $\mu$ g/ml) oil. Mustard and sunflower oil revealed least DPPH radical scavenging activity with IC<sub>50</sub> value of 1858.89  $\mu$ g/ml and 1557.63  $\mu$ g/ml respectively. In sterculia, groundnut and soybean oil, there is no significant differences whereas sunflower and mustard oil showed significant differences in DPPH activity.

#### 219

#### 3.5.2. ABTS Radical Scavenging Activity

ABTS radical scavenging activity of all the five oils were represented in IC<sub>50</sub> ( $\mu$ g/ml) as shown in Figure 1. Sunflower oil showed highest ABTS<sup>-+</sup> scavenging activity with significantly low IC<sub>50</sub> of 19.39  $\mu$ g/ml. Soybean oil showed least antioxidant activity with highest IC<sub>50</sub> of 237 $\mu$ g/ml, followed by sterculia (IC<sub>50</sub> of 220  $\mu$ g/ml), mustard (IC<sub>50</sub> of 199  $\mu$ g/ml) and ground nut (IC<sub>50</sub> of 169  $\mu$ g/ml). No significant differences were observed in ABTS activity of groundnut, mustard, sterculia and soybean except sunflower.

#### 226 **3.5.3. NO Radical Scavenging Activity**

NO radical scavenging activity of all the five oils expressed in IC<sub>50</sub> (µg/ml) is given in Figure 1.
 Sterculia oil exhibited highest NO radical scavenging activity with low IC<sub>50</sub> value of 114.98

 $\mu$ g/ml, followed by mustard (121.97  $\mu$ g/ml), groundnut (123.14  $\mu$ g/ml), soybean (230.24  $\mu$ g/ml) and sunflower (266.64  $\mu$ g/ml) oils. NO radical scavenging activity did not show any 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 DMSO on MCF-10A cell line. From MTT assay it was revealed that the sterculia oil did not 235 show any cytotoxicity on both normal and cancer cell lines. On cancerous cell lines namely 236 237 BT-474 and AU-565, sterculia oil at 10  $\mu$ g/ml concentration exhibited 92.91% and 90.07% survivability of cells respectively, at 20 μg/ml concentration 92.38% and 85.12%, at 40 μg/ml 238 239 concentration 89.47% and 80.64% and at 80  $\mu$ 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 oil at 10 µg/ml concentration showed 94.40% and 92.08% survivability of cells respectively 241 whereas at 20 µg/ml concentration 92.62% and 85.35%, at 40 µg/ml concentration 86.61 and 242 243 84.42% and at 80  $\mu$ g/ml concentration 80.56% and 76.89% survivability of cells were detected. The control experiment was done with DMSO on all cell lines in the same 244 245 concentrations as the tested samples. Minor reduction in survivability of cells was observed due to the presence of DMSO as the solvent. 246

#### 247 **4. Discussion**

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

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

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

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

Unsaturated fats helps to reduce the risk of heart disease and lower the cholesterol as they 276 277 replace saturated fats in the diet [EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 2010]. Along with this the data also shows that PUFA are present in higher quantity than the 278 279 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-6 fatty acids that the body needs for proper brain function and cell growth.Omega-3 fats lower 281 282 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. sterculic acid [namely 8-(2-Octacyclopropen-1-yl) octanoic acid] was found in the Sterculia 284 285 seed oil (Kale et al, 2011; Vipunngeun and Chanida, 2009). Sterculic acid is a potent natural 286 product to fight against obesity by suppressing a bodily enzyme associated with insulin resistance, which could indirectly help with reducing belly fat (Bao et al, 2003). It is also known 287 288 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 (Ortinau, et al, 2013). This sterculic acid directly inhibits SCD activity, possibly by a turnover-291 reaction, without affecting the processes required for 292 dependent adipocyte differentiation, scd gene expression or SCD protein translation (Gomez et al, 2003). So 293 294 Sterculia oil has a promise to act to reduce some factors causing obesity.

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

298 No significant differences in IC<sub>50</sub> values among the oils were observed as measured by 299 NO radical scavenging activity. ABTS radical scavenging activity showed significant difference

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

Furthermore, sterculia oil did not reveal any cytotoxic effect even at 40µg/ml 302 303 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 consumption, however, further studies are to be performed in order to ensure the same. All 305 306 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).

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

#### 319 **5.** Conclusions

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

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

333 **Conflicts of Interest** 

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

#### 336 Authorship Contribution Statement

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

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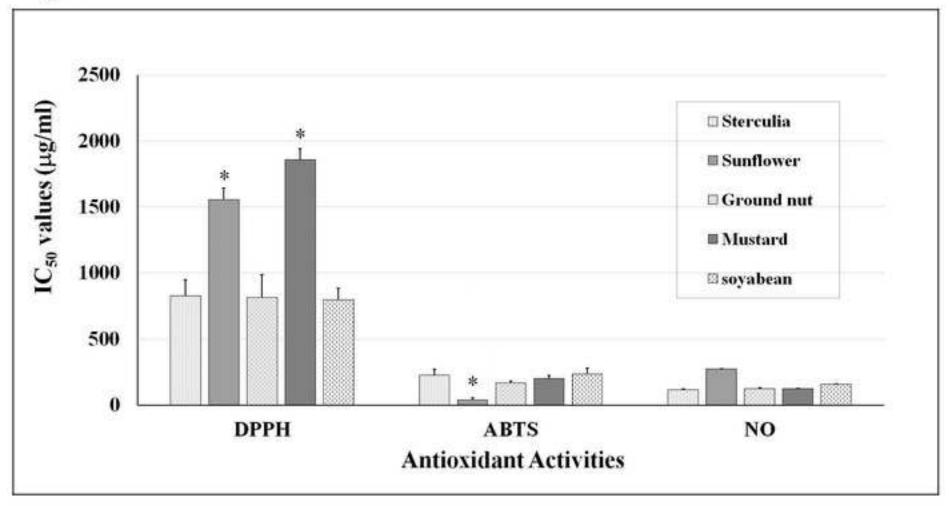
#### 484 **Figure and Captions**

485 Figure 1. Antioxidant activities of all the five oils (namely Sterculia, Sunflower, Groundnut,

- 486 Mustard and Soybean) expressed in  $IC_{50}$  (µg/ml). All the data are mean ± SD of triplicate
- 487 measurements. Bars with '\*' are significantly different. IC<sub>50</sub>, 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.
- Table 1. Proximate and mineral compositions of *Sterculia foetida, L.* seeds along with
   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 of496 respective oils.
- Table 3. Iodine value and oxidation status of five seed oils namely Sterculia, Sunflower,
   Groundnut, Soybean and Mustard.

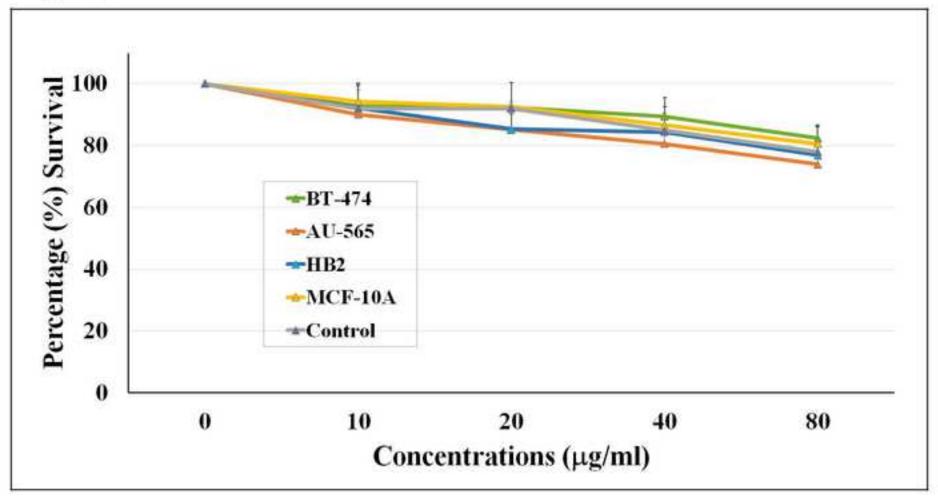


## Figure 1





## Figure 2



	Sterculia	Sunflower	Groundnut	Mustard	Soybean		
NUTRITIONAL COMPONENTS (g/100g)							
Oil Content	<b>58.7± 2.37</b> <sup>a</sup>	47.5± 2.22ª	49.8± 1.62 <sup>a</sup>	$\pm 1.62^{a}$ 31.4 $\pm 1.45^{b}$			
Lipid Content	58 .4± 4.52 <sup>a</sup>	51± 3.50 <sup>a</sup>	50± 6.52 <sup>a</sup>	36± 3.36 <sup>a</sup>	18.39± 4.52 <sup>b</sup>		
Protein Content	<b>38.43± 2.81</b> <sup>a</sup>	20.91± 3.08°	38.61± 3.02 <sup>a</sup>	28.8± 4.05 <sup>b</sup>	<b>37.69± 2.66</b> <sup>a</sup>		
Soluble Sugar Content	1.73±1.34°	2.54± 1.22 <sup>c</sup>	1.46± 1.26°	6.74±1.05 <sup>b</sup>	16.24±2.73 <sup>a</sup>		
Moisture Content	5.28± 3.22 <sup>b</sup>	7.18± 3.22 <sup>b</sup>	5.98± 2.01 <sup>b</sup>	9.12± 4.33 <sup>a</sup>	13.5± 2.44 <sup>a</sup>		
ALKALINE EARTH METALS (mg/kg)							
Magnesium	3436 ±4.51 <sup>b</sup>	3251±3.61 <sup>a</sup>	<b>590.33 ±6.55</b> <sup>a</sup>	<b>3040±72.11</b> <sup>a</sup>	2756.67±40.5		
Sodium	350.67±24.44 <sup>a</sup>	89.33±3.05 <sup>b</sup>	335±4.35 <sup>a</sup>	52.67±6.42 <sup>b</sup>	181±3.60 <sup>a</sup>		
Potassium	19858±345.5 <sup>b</sup>	6455.67±6.0 <sup>b</sup>	14500±2.01 <sup>a</sup>	6882±65.21 <sup>b</sup>	5293.33±15.6		
Calcium	590.33±4.51°	3251±3.61ª	2002.33±5.8 <sup>b</sup>	5218.33±7.6 <sup>a</sup>	3124±5.29ª		
HEAVY METALS (mg/kg)							
Copper	<b>16.67± 4.16</b> <sup>b</sup>	20±2.03 <sup>b</sup>	52.67±2.08 <sup>a</sup>	<b>38.67±1.15</b> <sup>a</sup>	48±3.0 <sup>a</sup>		
Lead	12.6± 3.05 <sup>b</sup>	10.33±1.52 <sup>b</sup>	44±2.35 <sup>a</sup>	<b>48.67± 8.14</b> <sup>a</sup>	38.33±5.50ª		
Manganese	10.6± 1.15 <sup>b</sup>	21±2.01 <sup>b</sup>	62±2.64 <sup>a</sup>	41.33±2.5 <sup>a</sup>	41±1.03 <sup>a</sup>		
Iron	38.67± 2.51 <sup>b</sup>	53.33±1.52 <sup>b</sup>	111±2.64ª	146.33±1.52 <sup>a</sup>	38.67±2.51 <sup>b</sup>		
Zinc	138.33±2.08ª	52.67±3.05 <sup>b</sup>	75±5.56 <sup>a</sup>	38.67±1.15 <sup>b</sup>	52.67±1.52 <sup>b</sup>		

 Table 1. Proximate and mineral compositions of Sterculia foetida seeds along with Sunflower,

 Groundnut, Mustard and Soyabean seeds.

Mean values  $\pm$  standard deviation for n = 3

\*Values (means $\pm$  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 andSoybean based on MDLC analyses of the FAME samples of respective oils.

Name of the fatty acid	Fatty acid content (mg/ml)					
Name of the fatty actu	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	$0.20 \pm 0.04$	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	$0.04 \pm 0.001$	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	$0.08 \pm 0.012$	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	$1.25 \pm 0.005$	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	

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

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

 Ground nut, Soyabean, Mustard.

The values are mean ± SD of three independent experiments

### **Declaration of interests**

 $\boxtimes$  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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On behalf of all the contributing authors, I, Suparna Mandal Biswas (corresponding author of the article), hereby decleare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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