

Possibility of Using Golden Shower (*Cassia Fistula*) and Poinciana (*Delonix regia*) Seeds Oil as Non-Conventional Feedstocks for the Production of Biodiesel in Egypt

Ahmed Mohamed Algharib^{1*}, Ahmed Fawzy Abd El Hakim²,
Haitham Ahmed El-Khamissi², Sam Mohamed El-Hamamsy²

¹ Environment and Bio-agriculture Department, Faculty of Agriculture, Al-Azhar University, 11884 Nasr City, Cairo, Egypt

² Biochemistry Department, Faculty of Agriculture, Al-Azhar University, 11884 Nasr City, Cairo, Egypt

* Corresponding author's email: aelghareb@gmail.com

ABSTRACT

The tree pods and seeds of *Cassia fistula* (CF) and *Delonix regia* (DR) were collected from the Faculty of Agriculture garden at Al-Azhar University in Cairo, Egypt, during the spring season of 2019. The physical and chemical aspects of pods and seeds were examined. The percentage of oil and fatty acid compositions were then investigated. The pod weights ranged from 61.34 g in DR to 89.29 g in CF, with pod lengths ranged from 42.26 cm (DR) to 62.64 cm (CF). In *Cassia fistula*, the seed weight per pod, the number of seeds per pod, and the weight of 100 seeds per pod were 12.29 g, 84, and 17.22 g, respectively; whereas in *Delonix regia*, they were 11.31 g, 23.5, and 34.25 g. The *C. fistula* had high levels of chlorophyll a and b, as well as total chlorophyll (1.016, 1.025, and 2.041 mg g⁻¹ DW), while *D. regia* recorded the lowest levels (0.513, 0.228, 0.741 mg g⁻¹ DW). The results also showed that the *C. fistula* leaves were also heavier than the *D. regia* leaves, weighing 14.96 g and 5.02 g fresh and dry weight for *C. fistula* and 10.06 g and 3.87 g fresh and dry weight for *D. regia*, respectively. The seeds of both plants were chemically tested, with percentages of Lipid, Moisture, Protein, Ash, Fibers, and Total Carbohydrates of 2.11, 10.79, 17.10, 4.95, 5.51, and 59.53 percent for *Cassia fistula* and 2.13, 6.52, 18.75, 0.37, 13.28, and 58.95 percent for *Delonix regia*, respectively. Eleven fatty acids were found in both plants seeds oil (lauric, myristic, palmitic, palmitoleic, stearic, oleic, eliadic, linoleic, linolenic, gondoic, and behenic acid). A slight variation was noted in linoleic acid, which was the major component of both oils ranging from 52.17 (CF) to 54.77% (DR). The prediction of the iodine values resulted in 97.6 and 98.8 for *Cassia fistula* and *Delonix regia*, respectively, which is a promising model for the production of biodiesel in the future.

Keywords: biodiesel, *Cassia fistula*, *Delonix regia*, linoleic acid

INTRODUCTION

The demand for energy is always increasing due to the world's growing population (Abraham et al., 2017). The stocks of fossil fuels will soon run out as a result of this constant demand for energy. As a result, there is a pressing need to find alternative and renewable energy sources (Atapour and Kariminia 2011). There are a variety of sustainable energy sources, including biodiesel, which has recently received a lot of attention (Abraham et al., 2017). Biodiesel (fatty acid

methyl esters) exhibits the fuel qualities close to petroleum diesel; however, it is distinguishable from it as an environmentally benign fuel due to its biodegradability, non-toxicity; oxygen content and non-sulfur content (Stamenkovic et al., 2014). The process of esterification is used to produce biodiesel from vegetable oils. The most common edible vegetable oils used as a feedstock for biodiesel manufacturing are sunflower, rapeseed, and soybean oils. However, there are numerous obstacles to biodiesel manufacturing, the most significant of which being the high cost

(Abraham et al., 2017). In order to reduce the cost of biodiesel manufacturing, alternative vegetable oils that are not used for food have to be sought, such as *Ricinus communis* (Kotb and Algharib 2016), *Cassia fistula* (Abraham et al., 2017), and *Delonix regia* (Adejumo et al., 2019), as well as waste cooking oils (Wang and Haiyan 2012). The golden shower tree (*Cassia fistula*) is an ornamental plant that is grown all over the world and used for ornamental purposes, but it has recently been utilized in Ayurvedic medicine to treat a variety of ailments (Amitabye et al., 2002). Thirumal et al., (2012) reported that *C. fistula* has been utilized in traditional medicine for a long time. The various parts of this tree are utilized to treat a variety of illnesses. It has been reported to possess various activities like anthelmintic, antibacterial, antifertility, antifungal, anti-inflammatory and antioxidant activities. These trees generate a lot of pods with brown seeds that are not used, and tons of dried seeds are thrown away every year (Chauhan et al., 2011). Many studies have recently indicating the possibility of using these seeds to produce oil, which may then be used to make biodiesel. According to Abraham et al., (2017), the oil obtained from *C. fistula* seeds could be used as a raw material for biodiesel manufacturing. The Poinciana tree (*Delonix regia*) is another attractive tree that produces pods with unutilized seeds (Alamu, 2015). In arid and semi-arid environments, the tree is grown primarily for its shade and to minimize soil erosion (Hugh, 2002). Okey and Okey (2013) demonstrated that the *D. regia* oil may be used as a viable feedstock for biodiesel production that can effectively be utilized in diesel engines. Adejumo et al., (2019), reported that the low saponification value and higher molecular weight of the *D. regia* oil will make it suitable for use as biodiesel feed stock seed. According to Adejumo et al., (2019), *D. regia* oil has a low saponification

value and higher molecular weight, which qualify it to for usage as a biodiesel feedstock. Therefore, the objectives of this study were to compare and evaluate the *C. fistula* and *D. regia* seeds in terms of morpho-chemicals characteristics to explore the potential possibility of using these trees as a low-cost and non-edible raw material for the production of biodiesel in Egypt.

MATERIALS AND METHODS

Plant Material: The *Cassia fistula* and *Delonix regia* seed pods were randomly collected from the trees located on the campus of Faculty of Agriculture, Al-Azhar University, Cairo, Egypt in spring 2019. The chemical characteristics of soil in which the trees grow were analyzed, as well as the water used to irrigate these trees. The results of the analysis are in Table 1 and Table 2.

Pods and seeds parameters: The pods (Fig. 1) were air-dried for around 100 days in laboratory and studied for their morphometric parameters (viz. pod length (cm), pod diameter (cm), pod volume (cm³), pod weight (g), seeds weight per pod (g), 100 seeds weight(g), and number of seeds per pod (Khan and Javed, 2012). Each pod was opened carefully. The ripe seeds (Fig. 2) were cleaned manually by removing the outer covering. The number of seeds was recorded from each pod and seeds were stored in dry bottles for further study. Then, 100 randomly selected seeds were weighed individually (Sokal and Rholf, 2012).

Chlorophyll pigments: The amount of Chlorophyll a, Chlorophyll b, and total Chlorophyll were calculated by using the methods of El-Serafy (2020). The pigments of Chlorophyll a, Chlorophyll b, and total Chlorophyll were extracted from leaf by using 80% acetone. The

Table 1. Soil chemical properties

| EC (dS/m) | pH 1:2.5 | SP | Anions mEq/L | | | | Cations mEq/L | | | |
|-----------|----------|------|-----------------|-------|-------------------------------|------------------------------|------------------|------------------|-----------------|----------------|
| | | | SO ₄ | Cl | HCO ₃ ⁻ | CO ₃ ⁻ | Ca ⁺⁺ | Mg ⁺⁺ | Na ⁺ | K ⁺ |
| 3.53 | 7.20 | 50.0 | 15.25 | 13.56 | 10.58 | - | 27.63 | 6.06 | 4.70 | 1.27 |

Table 2. Irrigation water analysis

| EC (dS/m) | pH 1:2.5 | Anions mEq/L | | | | Cations mEq/L | | | | Sodium Adsorption Ratio (SAR) (%) |
|-----------|----------|-----------------|------|-------------------------------|------------------------------|------------------|------------------|-----------------|----------------|-----------------------------------|
| | | SO ₄ | Cl | HCO ₃ ⁻ | CO ₃ ⁻ | Ca ⁺⁺ | Mg ⁺⁺ | Na ⁺ | K ⁺ | |
| 0.41 | 7.00 | 1.89 | 1.19 | 1.23 | - | 2.54 | 1.10 | 0.61 | 0.06 | 0.45 |

different optical densities were recorded at 470, 653 and 662 nm in spectrophotometer.

Seeds oil extraction

Abraham *et al.* (2017), reported that maximum yield of oil was obtained with hexane for the reaction time of 3.5 h and is considered as the best solvent for this work. The ripe seeds were cleaned manually by removing the outer covering and dried in the sunlight prior to extraction. The dried seeds (400 gram) were ground into powder using a laboratory mill (30 sec at 25°C, Ika-Werke M20 Analytical Mill, Staufen, Germany). The oil extraction was carried out with 50 grams of the milled seeds with 250 ml of hexane as a solvent for the reaction time of 3.5 h, using a Soxhlet extractor (Qin *et al.*, 2010).

Chemical Composition Analysis

In order to investigate the chemical composition for each of the studied plants, *Cassia fistula* and *Delonix regia*, Near-InfraRed (NIR) Spectroscopy equipment, model DA1650, produced by FOSS, Corporation was utilized at the central laboratory, Faculty of Agriculture of Al-Azhar University, Taha *et al.*, 2016.

Lipid Extraction and Methylation: By using the Soxhlet extraction method, the Petroleum ether (82.2 g/Mol, 80°C) was applied to extract the oil from the dried, powdered seeds, which was then concentrated using a rotary evaporator. A methanol/acetyl chloride solution was used to trans methylate about 50 μL of each sample of the extracted oil, as used by Masood *et al.*, 2005. Then, the Gas chromatography (GC) was used



Figure 1. *Delonix regia* pods (a), and *Cassia fistula* pods (b)



Figure 2. *Delonix regia* seeds (a), and *Cassia fistula* seeds (b)

to identify the resulted fatty acid methyl esters (FAMES).

Gas Chromatography: On the Trace Thermo Gas Chromatography system, methylated oil in hexane (1.0 μL) was gas chromatographed. With helium carrier gas (at a flow rate of 1 cm^3/min), the components of FAMES were eluted. The TG-1MS Guard GC columns (with a length of 30m, an i.d. of 0.25 mm, and a particle size of 25 μm) were utilized. The oven was preheated to 60°C for 3 minutes before ramping to 250°C at a rate of 10°C/min for 15 minutes. The samples were injected at 230°C, while the detector was maintained at 250°C.

Prediction of the Iodine Value (IV): The following formula was used to determine the iodine levels regarding the contents of the FAMES:

$$IV = xC1 + yC2 + zC3 \quad (1)$$

where: *C1*, *C2*, and *C3* are the percentages of unsaturated fatty acids,

x, *y*, and *z* are coefficients (*x* = 1, *y* = 1.5, and *z* = 2.62), Goldson Barnaby *et al.*, 2016a.

Statistical Analysis: The results were expressed as means \pm S.D. Comparison of means was done using the least significant difference of (at $P < 0.05$) by one-way Analysis of Variance Gen Stat software.

RESULTS AND DISCUSSION

Chlorophyll a, Chlorophyll b, and Total chlorophyll of *Delonix regia* and *Cassia fistula* :

Chlorophyll is the green pigment present in plants playing a vital role in photosynthesis which absorbs the light from the Sun and uses its energy to synthesize carbohydrates from CO_2 and water (Gaykhe and Kadam, 2017). The data of Chlorophyll a, Chlorophyll b, and Total chlorophyll of *Delonix regia* and *Cassia fistula* are presented in table 3. The obtained results showed that the chlorophyll a and b contents, and total chlorophyll were high in *C. fistula* (1.016, 1.025, and 2.041 mg g^{-1} dry weight), while they were low in *D. regia* (0.513, 0.228, 0.741 mg g^{-1} dry weight). These results are in agreement with Thawale *et al.*, (2011), who reported that total chlorophyll for *Cassia fistula* was higher than *Delonix regia* in three sites (4.811, 2.331, and 5.014 mg g^{-1} dry weight) for *C. fistula* and (4.215, 4.014, and 3.341 mg g^{-1} dry weight) for *D. regia*. Miria and Khan (2013), also found that the Chlorophyll content of *Delonix regia* (9.07 $\mu\text{mol m}^{-1}$) was lower than *Cassia fistula* (31.09 $\mu\text{mol m}^{-1}$).

Quantitative parameters

The data collected on various quantitative parameters of *C. fistula* and *D. regia* mature pods and their seeds are presented in Table 4.

Table 3. Chlorophyll a, Chlorophyll b, and Total chlorophyll

| | Chlorophyll a | | | Chlorophyll b | | | Total chlorophyll | | |
|-----------------------|---------------|-------|------|---------------|-------|------|-------------------|-------|------|
| | M | | SD | M | | SD | M | | SD |
| <i>Delonix regia</i> | 0.513 | \pm | 0.25 | 0.228 | \pm | 0.13 | 0.741 | \pm | 0.38 |
| <i>Cassia fistula</i> | 1.016 | \pm | 0.04 | 1.025 | \pm | 0.17 | 2.041 | \pm | 0.14 |

* The data represent mean \pm SD of three replicates, ** M= Mean, SD = Standard deviation.

Leaves fresh and dry weights. Fresh and dry weights of leaves for *C. fistula* and *D. regia* are presented in table 4. The results showed that the leaves of *C. fistula* were heavier than the *D. regia* leaves (14.96 g and 5.02 g fresh and dry weight) for *C. fistula* and (10.06 g and 3.87 g fresh and dry weight) for *D. regia*.

Pod length and Pod weight. The pods in *Delonix regia* were shorter in length than the pods in *Cassia fistula*, as the pods in *D. regia* was 42.26 cm while the *C. fistula* was 62.64 cm (Table 4). On the other hand, the *C. fistula* pods were heavier than the pods of *D. regia*, as the weight of the pod in *C. fistula* was recorded at 89.29 g, while in *D. regia* it was 61.34 g. Khan & Zulfiqar (2013), showed that the pods of *D. regia* were 30–60 cm long, and the average weight of air dried pods was 61.67 g.

Seed weight/pod. The seeds of *C. fistula* recorded the heaviest weight (12.29 g), while the seeds of *D. regia* were the lightest (11.31 g).

Number of seeds per pod. Akinyede and Amoo (2009) indicated that *C. fistula* seeds are a good source of human food, as the seeds contain proteins (26%) and carbohydrates (50%). Seeds may be used in food formation if phytic acid is removed. The data in Table 4 indicated that the number of seeds in the pods in the *Cassia fistula* plants was higher (84), than that of the *Delonix regia* pods (23.56).

100 seeds weight. Although *Cassia fistula* recorded the highest number in seeds per pod, they were lighter than *Delonix regia* seeds, as the weight of one hundred grains was recorded 17.22 g in *C. fistula* and 34.25 g in *D. regia*. Seed size variation within species and individuals is common (Halpern, 2005). The variation in seed size may be the result of myriad of factors (Wulff, 1986). Winn (1991) has suggested that plants may not have the capability of producing a completely uniform seed weight simply as a result of

variations in resource availability (e.g., soil moisture during seed development). Seed size is significantly reduced under moisture stress in mature trees of walnut (Martin *et al.*, 1980). Seed weight is said to be the direct function of precipitation (moisture availability) and monthly precipitation is reported to explain around 85% of the total variation in seed weight in Wyoming sage brush, *Artemisia tridentate* (Busso and Perryman, 2005).

Chemical Composition Analysis

The chemical composition of the *Cassia fistula* and *Delonix regia* seeds was shown in table (5) and Fig. (3). The chemical composition analysis for each of the studied plants was performed in triplicate. None of the studied plants, for each of the examined parameters, resulted in significant differences. The highest component of the *C. fistula* and *D. regia* seeds was the carbohydrates, by the average of 59.53 and 58.95 respectively, which differed in the starch percentage reaching 23.34 for *C. fistula* and 30.47 for *D. regia*. Lesser difference was found in lipids reaching 2.11% and 2.13% of *Cassia fistula* and *Delonix regia* seeds, respectively. The *Cassia fistula* seeds were higher in moisture content at 10.79%, while the *Delonix regia* contained 6.52%. The protein contents in *D. regia* were 18.75%, while it was 17.10% in *C. fistula*. The fiber contents showed the highest difference to be 13.28% for *D. regia* and 5.51% for *C. fistula*. Another difference was in ash percentage in *C. fistula* at 4.95%, while only 0.37% in *D. regia*. The previous investigations on the *Delonix regia* and *Cassia fistula* seeds by Shrivastava *et al.*, 2015, Oyedeji *et al.*, 2017, approximately matched the present results on the moisture, fiber, protein, and ash parameters. In turn, the data on the other parameters differs from these results, due to different factors, such as species characteristics

Table 4. Morphometric parameters of leaves, pods, number of seeds per pod and seeds weights in *Cassia fistula* and *Delonix regia*

| | <i>Cassia fistula</i> | | | <i>Delonix regia</i> | | |
|-------------------------|-----------------------|---|-------|----------------------|---|------|
| | M | ± | SD | M | ± | SD |
| Leaves fresh weight (g) | 14.96 | ± | 1.26 | 10.06 | ± | 0.28 |
| Leaves dry weight (g) | 5.02 | ± | 0.69 | 3.87 | ± | 0.16 |
| Pod length (cm) | 62.64 | ± | 2.88 | 42.26 | ± | 3.17 |
| Pod weight (g) | 89.29 | ± | 8.28 | 61.34 | ± | 5.63 |
| Seeds weight / pod (g) | 12.29 | ± | 2.18 | 11.31 | ± | 1.69 |
| No. of seeds/ pod | 84 | ± | 11.05 | 23.56 | ± | 3.95 |
| 100 seeds weight (g) | 17.22 | ± | 0.34 | 34.25 | ± | 1.04 |

* The data represent mean ± SD of three replicates, ** M= Mean, SD= Standard deviation.

or planting conditions. These results refer to the good nutritional, medicinal, and economical advantages for each of the studied plants, as mentioned by Akinyede *et al.*, 2009, Sharma *et al.*, 2020. Early reports explored the potential of the near-infrared spectroscopy techniques for identifying the chemical composition of plant biomass, to distinguish high-quality feedstock to produce biofuel (Liu *et al.*, 2010; Penning *et al.*, 2014; Sykes *et al.*, 2009; Vogel *et al.*, 2011). The broad connections between biomass quality and biofuel production have been the subject of many reviews (Collard and Blin 2014; Lin *et al.*, 2015; Tanger *et al.*, 2013). The variations in the biomass components were considered the plant species, the plant organs, and the developmental stages. Vogel *et al.*, (2011), deduced in addition to most of the cell wall structure and the intrinsic moisture, The dry biomass of biofuels comprises around 6% proteins, about 5% non-structural carbohydrates, and ash in the range from 0.4% to 14%.

Fatty Acid Profile and predicted Iodine Value (IV)

By comparison, the *Delonix regia* seeds resulted in a higher percentage of the lipids average, by 2.13%. In turn, the seeds of *Cassia fistula*

contained an average of 2.11%, as shown in table (5) and Fig. (3). During the investigation, the gas chromatography (GC) technique was used to identify the fatty acid profile of the resulted fatty acid methyl esters (FAMES) from the extracted oils. The linoleic acid, unsaturated fatty acid, was found at the highest plentiful supply in the tested oils, reaching 54.77% in *Delonix regia*, while the *Cassia fistula* seeds contained about 52.17%. Next, the fatty acid palmitic acid, saturated fatty acid, was found to be higher in *Cassia fistula*, representing 19.08% more than *Delonix regia* which contained 13.94%. Then, the fatty acid Oleic, unsaturated fatty acid, recoded 17.85% in *Cassia fistula* seeds, while it was 15.48% in *Delonix regia*. The least share in the fatty acid profile was of stearic acid, saturated fatty acid, which represented 7.12% and 12.22% in *Cassia fistula* and *Delonix regia*, respectively. Moreover, traces of many other fatty acids were found in the extracted oil from the studied plants, by less than 2%, as shown in table (6) and Fig. (4). These results nearly resemble those were previously found by Goldson Barnaby *et al.* [2016a,b].

Meanwhile, the percentage of unsaturated fatty acid was 70.99 and 71.47 in *Cassia fistula* and *Delonix regia*, respectively. On the other hand, the saturated fatty acids represent only 28.75%

Table 5. Average Values for Chemical Composition Analysis for both of *Cassia fistula* and *Delonix regia*.

| | Lipid % | Moisture % | Protein % | Ash % | Fiber % | Total Carbs. % | Starch % |
|-----------------------|---------|------------|-----------|-------|---------|----------------|----------|
| <i>Cassia fistula</i> | 2.11 | 10.79 | 17.10 | 4.95 | 5.51 | 59.53 | 23.34 |
| <i>Delonix regia</i> | 2.13 | 6.52 | 18.75 | 0.37 | 13.28 | 58.95 | 30.47 |
| L.S.D. (0.05) | 0.076 | 0.629 | 0.205 | 0.952 | 0.980 | 0.361 | 0.200 |

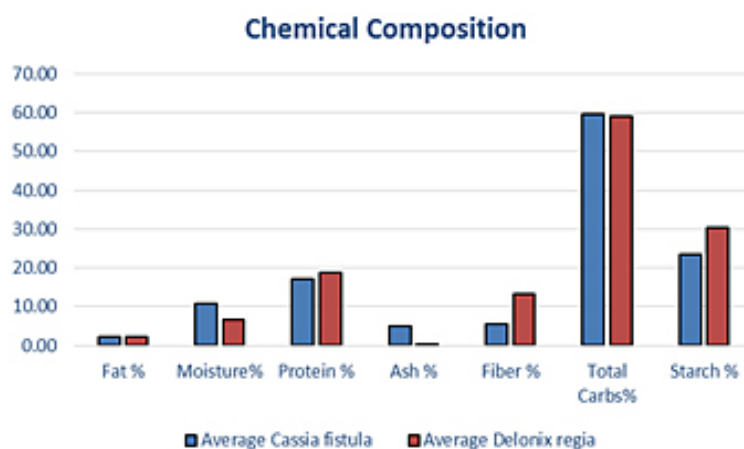
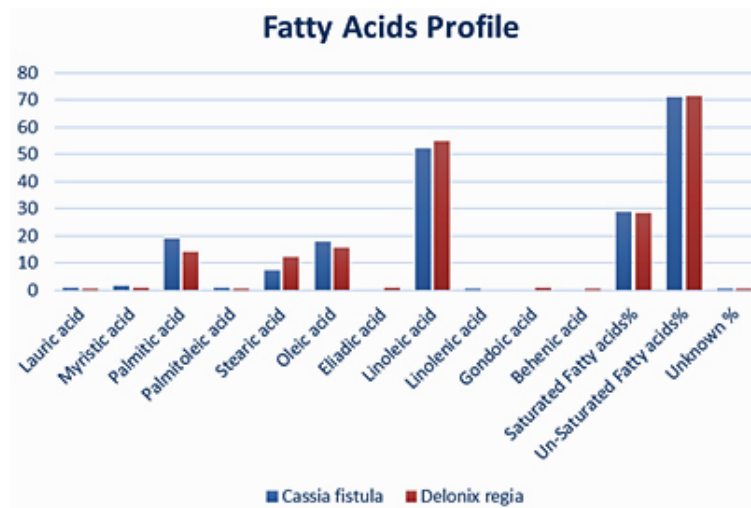


Figure 3. Representation of the Chemical Composition Analysis for both of *Cassia fistula* and *Delonix regia*

Table 6. Fatty acids profile for each of the studied plant seed oil.

| Fatty acid | | <i>Cassia fistula</i> | <i>Delonix regia</i> |
|-----------------------------|-------------|-----------------------|----------------------|
| | | % | % |
| Lauric acid | C12 | 0.91 | 0.19 |
| Myristic acid | C14 | 1.64 | 0.6 |
| Palmitic acid | C16 | 19.08 | 13.94 |
| Palmitoleic acid | C16:1 | 0.62 | 0.28 |
| Stearic acid | C18 | 7.12 | 12.22 |
| Oleic acid | C18:1 cis | 17.85 | 15.48 |
| Eliadic acid | C18:1 Trans | ND | 0.94 |
| Linoleic acid | C18:2 | 52.17 | 54.77 |
| Linolenic acid | C18:3 | 0.35 | ND |
| Gondoic acid | C20 | ND | 0.95 |
| Behenic acid | C22 | ND | 0.38 |
| Saturated Fatty acids% | | 28.75 | 28.28 |
| Un-Saturated Fatty acids% | | 70.99 | 71.47 |
| Unknown % | | 0.26 | 0.25 |
| Total % | | 100 | 100 |
| Predicted Iodine Value (IV) | | 97.642 | 98.855 |

**Figure 4.** Fatty acids profile representation of the studied plants seed oil

and 28.28%, for both *C. fistula* and *D. regia*, respectively. Therefore, the prediction of the iodine values resulted in 97.635 and 98.855 for *Cassia fistula* and *Delonix regia*, respectively, as shown in table (6) and figure (2). The European Committee for Standardization (ECS) described the most common needs of the FAMES as a biodiesel oil, to fall in the range of 100 up to 120 of the Iodine Value (IV). The iodine value measures are in place to keep track of the natural inclination of fuel to oxidize. Higher iodine levels are linked to increased oxidation, Noor et al., 2018, Tsoutsos et al., 2019.

CONCLUSIONS

The results of the current study indicate the possibility of using *Delonix regia* and *Cassia fistula* plants as non-traditional sources for biodiesel production, especially in dry areas such as Egypt.

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