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Original Article

Biologically active compounds in *Delonix regia* (Flame of forest) seed oil extracted by hexane

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Abstract

Delonix regia is an underexploited plant with oil-rich seeds. This study characterized the bioactive constituents in the seed oil with a view to ascertaining its usefulness in replacing expensive conventional oils in industrial applications. Identification and quantification of the compounds were done with gas chromatography with flame ionization detector (GC-FID) after extraction using n-hexane. The main bioactive contents per 100g oil were tocopherols (53.10 mg), tocotrienol (1.64 mg), terpenoids (0.461 mg), phospholipids (378.11 mg), and sterols (507.42 mg). Sitosterol (310.63 mg), campesterol (105.81 mg), and stigma-sterol (78.31 mg) dominated the sterols while phosphatidylcholine (249.43 mg) and phosphatidylinositol (81.56 mg) were the major phospholipids. The major tocols were α-tocopherol (3.93 mg) and ɣ- tocopherol (48.83 mg), whereas ɣ-tocotrienol (1.08 mg) was the major tocotrienol. Terpenoids including taraxerol, α-amyrin, β- amyrin, luperol and bauerenol acetate were quantified in trace amounts. It was concluded that the *D. regia* seed oil could contribute to novel research applications in food, cosmetics, pharmaceutical, and various other non-food industries.

Keywords: bioactive compounds, *Delonix regia*, GC-FID, underutilised seed oil

1. Introduction

The growth trend in world population presents a challenge to food security, with potential production shortages and unpredictable prices. Therefore, there is a dire need for increased food production to keep up with the demand (Fernandez-Arche, Montserrat-de la Paz, Marin-Aguilar & Garcia-Gimenez, 2014). The dietary and functional roles of fats in human diets coupled with increases in prices of conventional oils necessitate characterizing more vegetable oils for advanced uses of their components, as well as of their byproducts, to meet the ever-increasing demand for vegetable oils, especially from underutilized seed legumes (Nehdi *et al.*,2012; Oyedeji, Azeez & Osifade, 2017). Although bioactive food constituents are not nutritionally critical to life, they however have medicinal properties to improve health, and pharmaceutical and food industries are showing increasing interest in obtaining and characterizing new bioactive compounds that can be incorporated into drugs, or used as practical food ingredients or nutraceuticals (Ellwood *et al.*, 2014; Gonzalez-Aguilar *et al.*, 2013).

Delonix regia tolerates well most soil types and is widely spread in Sudan and Sahel savannah. The trunk of *D. regia* has served as wood for the construction industry, and as fuel for domestic purposes (Bichi, 2012). *D. regia* seeds are underexploited sources of vegetable oil with only a few studies describing their physicochemical characteristics and potential uses. They contain an appreciable amount of oil (approximately 17%) and are particularly rich in unsaturated fatty acids (Barnaby, Reid, Rattray, Williams & Denny, 2016; Oyedeji, Azeez & Osifade, 2017). A better understanding of the composition of underutilised seeds offers new prospects for their cultivation and agronomy; thus, this study was aimed at conducting detailed analyses of the bioactive components of

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the seed oil, with a view to create a database stimulating further work on them.

2. Materials and Methods

2.1 Plant materials

Seeds of *D. regia* were collected from the botanical garden of the Department of Science Laboratory Technology, The Federal Polytechnic, Ilaro, between March and June 2018. Seed samples were identified at Forestry Research Institute of Nigeria (FRIN) with voucher number FHI 109807.

2.2 Sample preparation

2.2.1 Oil extraction

The extraction was done with n-hexane as previously described by (Oyedeji, Azeez & Osifade, 2017) and a rotary evaporator (RE300, Bibby Scientific Ltd., United Kingdom) was used for solvent removal at reduced pressure, while remaining traces of n-hexane were removed using nitrogen gas.

2.2.2 Analytical methods

Tocopherols and tocotrienols in the seed oils were determined as described by Du and Ahn (2002). Sterols and terpenoids contents of the oil were evaluated using the modified methods of AOAC (Official Methods of Analysis [AOAC], 1996a, 1996b). The method of Raheja, Kaur, Singh, and Bhatia (1973) was used for the analysis of phospholipids. An HP 6890 GC-FID (Agilent Technologies, Santa Clara, United States) system was used for the analyses with split injection in 20:1 ratio and detector temperature of 300°C. The inlet temperature was maintained at 290 °C with helium as the carrier gas for tocopherols and tocotrienols, while nitrogen was the carrier gas for sterols, terpenoids and phospholipids. HPINNOWax column (30 m x 0.25 mm x 0.25 µm) was used for sterols and terpenoids analyses while HP-5ms UI with the same dimensions was used for the studies of tocopherols, tocotrienols and phospholipids. The oven program had an initial temperature of 180 °C for 5 min. The first ramp was at 8 °C/min to 260 °C and the second was at 2 °C/min to 280 °C, which was held constant for 13 min. The flow rate and hydrogen pressure were 2.5 mL/min and 22 psi, respectively, while data acquisition was performed with HP ChemStation Rev. A 09.01 [1206] software.

3. Results and Discussion

3.1 Tocol (Tocopherol and Tocotrienol) composition

The *D. regia* seed oil contains all the four isomers of vitamin E in various proportions. Gamma-tocopherol is the most abundant (91.96%) which is higher than the 10.5, 58.0, 46.0 and 88% reported for Brazilian nut (*Bertholletia excelsa*), olive, walnut, and soya bean oils, respectively (Chunhieng *et al*., 2008; Nehdi *et al*., 2012). The total concentration of α- and γ- tocopherols in the oil sample was 52.76 mg/100 g, while the total tocol content was 54.74 mg/100 g (Table 1). The tocopherol content of the sample oil (53.10 mg/100g) is higher than 42.54 and 33 mg/100 g of total tocopherol reported by Nehdi *et al.* (2012) for cold pressed olive and palm kernel oils, respectively. The value is close to the range reported by Bazongo *et al*. (2014) for coconut oil (55 mg/100 g) and *Lannea microcarpa* seed oil (57.86 mg/100 g). A high tocopherol content in vegetable oils is noted for conferring oxidative stability and antioxidant activity (Bazongo *et al*., 2014). Tocopherols have also been reported to modulate physiological processes associated with inflammation, skin barrier homeostasis, and wound healing, and are well noted for their photo protective effects (Lin, Zhong & Santiago, 2018; Styrczewska, Zuk, Boba, Zalewski & Kulma, 2019). Sodium dl-α-tocopheryl-6-O-phosphate is a water-soluble derivative of α-tocopherol that enhances the gene expression for differentiation markers and synthesis of ceramide (Lin *et al*., 2018). Tocotrienols, though more potent in antioxidant activity, are less abundant in plant products compared to tocopherols, and this is the trend also in this study (Hovarth *et al*., 2006; Serbinova & Packer, 1994). Due to the high levels of γ-tocopherol, the oil could find application in cosmetic and pharmaceutical formulations and add value to and improve stability of those products, due to the inherent antioxidant capacity in addition to the emollient effects.

Table 1. Tocopherol, tocotrienol, and terpenoid levels in the seed oil of *D. regia*

Group of compounds	Compound	Amount (mg/100g)
Tocopherols	α-tocopherol	3.93
	γ-tocopherol	48.83
	β-tocopherol	1.10 x 10^{-4}
	δ-tocopherol	3.35 x 10^{-1}
Total		53.10
Tocotrienol	α- tocotrienols	4.87 x 10^{-1}
	γ-tocotrienol	1.08
	β-tocotrienol	7.06 x 10^{-2}
	δ-tocotrienol	2.11 x 10^{-5}
Total		1.64
Terpenoids	Taraxerol	3.66 x 10^{-4}
	α-amyrin	1.26 x 10^{-2}
	β-amyrin	4.41 x 10^{-1}
	Luperol	1.63 x 10^{-3}
	Bauerenol acetate	5.76 x 10^{-3}
Total		4.61 x 10^{-1}

3.2 Terpenoid composition

The results in Table 1 indicate that terpenoids, namely taraxerol, α-amyrin, β-amyrin, luperol, and bauerenol acetate, were present in D. *regia* seed oil in trace amounts (4.61 x10^{-1} mg/100g). Terpenoids are commonly found in small fractions as plant oil constituents. They contain an array of molecules useful in many biological processes, ranging from cell proliferation to migration and collagen build-up. They have been reported to improve wound healing by enhancing tissue repair within a short period, together with mitigating the production of reactive oxygen species (ROS) in the wound microenvironment (Lin *et al*., 2018). α- and β-amyrin reported in this study have been isolated from *Alstonia boonei* stem bark, and have profound anti-inflammatory activities in rodents (Okoye *et al*., 2014). Ebajo, Shen, Consolacion and Ragasa (2015) also isolated these terpenoids from *Hoya multiflora* (shooting-star hoya) stem and leaves but did not report any biological activity of the isolates. De Amorim, Godinho, Archanjo and Grael (2016), however, isolated the same compounds from the leaves of *Pseudobrickellia brasiliensis* that are used as painkillers and for treating inflammation. The presence of terpenoids in the oil suggests that it could be useful as an analgesic and anti-inflammatory agent, as has been previously reported.

3.3 Phospholipids

The *D. regia* seed oil contained 378.11 mg/100 g phospholipids (Table 2.). The oil is rich in phosphatidyl choline with 249.43 mg/100 g accounting for 65.97% of total phospholipids. Phosphatidylinositol and phosphatidylethanolamine were the next most abundant. Phospholipids have been established to improve the antioxidant properties of oils owing to the synergistic association between them and tocopherols (King, Boyd & Sheldon, 1992; Lin *et al*., 2018). Lysophosphatidylcholine, phosphatidylcholine, phosphatidyl ethanolamine, phosphatidylserine, phosphatidylinositol in a decreasing order enhance the oxidative stability and reduce the loss of polyunsaturated fatty acids (King *et al*., 1992). The seed oil of *D. regia* is rich in the aforementioned phospholipids. Phospholipids through their phosphate groups chelate pro-oxidant metals, act as chemical absorptivity enhancers, and as an oxygen barrier between oil and air interfaces (Berton-Carabin, Ropers & Genot, 2014; Budilarto & Kamal-Eldin, 2015; Kurutas, 2016). Phosphatidylcholine and phosphatidylserine have respectively been reported for treating liver conditions and the prevention of brain deterioration (Reichert, 2002). However, phospholipids have been reported to increase oil loss during neutralisation, along with other problems such as a decrease in the bleaching efficiency, filtration, production of off-flavors and darkening of oil after deodorisation (Ghazani & Marangoni, 2013). The removal of phospholipids, therefore, improves the oils' smell and taste characteristics, stability, and its organoleptic features (Popa, Babeanu, Popa, Nita, & Dinu-Parvu). It therefore follows that for *D. regia* seed oil to be suitable for cooking and other food purposes, it is necessary to remove its phospholipid contents.

3.4 Sterols

Sterols per 100 g of the sample seed oil were sitosterol (310 .63 mg), campesterol (105.81 mg), stig-masterol (78.31 mg) and Δ^5–avenasterol (12.68 mg). Sitosterol is the most prevalent and accounted for more than 60%. Other steroids found in trace quantities in the oil include cholesterol, cholestanol and ergosterol. The total sterol content of *D. regia* seed oil, as detailed in Table 2, was 507. 42 mg/100 g. Campesterol and stigmasterol in the oil exceeded the 4% acceptable limit in total sterols content (Al-Ismail, Alsaed, Ahmad, & Al-Dabbas, 2010). The total sterol contained in the sample seed oil (507.42 mg/100g) is higher than those reported for Tunisian olive oil (146.75−247.68 mg/100g) and for the acorn fruit oil (204.0–248.0 mg/100 g) that have been adjudged good in nutritional characteristics (Al-Rousan *et al*., 2013; Kammoun & Zarouk, 2012). Sterols from plants are critical constituents of cellular membranes, known for regulation of fluidity and permeability (Hartman, 1998; Schaller, 2003). Sterols possess effective ability to lower blood serum cholesterol and have been included in many cholesterol-lowering drugs for treating hypercholesterolemia and diabetes (Law, 2000; Ostlund, Racette, & Stenson. 2002). The consumption of vegetable sterols has been associated with a lower risk of myocardial infarction, lowering LDL cholesterol even when combined with non-fat matrices. Additionally, they can modulate biomarkers of oxidative stress and inflammation and moderate the development of atherosclerosis. Campesterol, β-sitosterol and stigmasterol have antitumor properties essential for treating breast, colon and prostate cancers, while stigmasterol is essential for cell proliferation (Hartman, 1998). This is connected to their ability to inhibit cancer cell tumors, and apoptosis (Fabrikova *et al*., 2019). From the foregoing, *D. regia* seed oil could be deployed in the delivery of synthesised anti-cancer nanoparticles, and drugs to enhance their potency.

Table 2. Phospholipids and sterol levels in the seed oil of *D. regia*

Group of compounds	Compound	Amount (mg/100g)
Phospholipids	Phosphatidyl	
	-ethanolamine	39.10
	-choline	249.43
	-serine	5.33
	-insitol	81.56
	Lysophosphatidyl choline	2.69
Total		378.11
Sterols	Cholesterol	6.93×10^{-6}
	Cholestanol	4.76×10^{-4}
	Ergosterol	1.93×10^{-5}
	Campesterol	105.81
	Stig-masterol	78.31
	Δ^5-avenasterol	12.68
	Sitosterol	310.63
Total		507.42

4. Conclusions

This study has revealed the richness of *D. regia* seed oil in tocopherols, tocotrienols, terpenoids, phospholipids and sterol, all natural biologically active constituents that could be of great pharmacological potential, if exploited. The use of this oil, either directly or through various food products, could inhibit several diseases including coronary disease and serve as antioxidant and anti-inflammatory functional ingredient. These findings should make the oil of interest for further study concerning toxicity and safety for human consumption or for non-food applications.

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