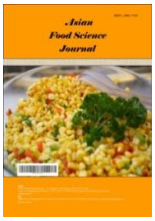

Asian Food Science Journal

17(2): 43-49, 2020; Article no.AFSJ.57770
ISSN: 2581-7752

Studies on *Allamanda cathartica* Seed Oil: A Novel Source of Plant Oil Substitute for Industrial Processing

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Authors' contributions

This work was carried out in collaboration among all authors. Author GOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SGO and FDA managed the analyses of the study. Authors MAS and MMO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2020/v17i230190
Editor(s):
(1) Dr. Olorunjuwon Omolaja Bello, University of Medical Sciences, Nigeria.
Reviewers:
(1) Gabriel Ribeiro Carvalho, Federal University of Lavras, Brazil.
(2) Li Cong, Northwest University, USA.
Complete Peer review History: http://www.sdiarticle4.com/review-history/57770

Original Research Article

Received 10 April 2020
Accepted 13 June 2020
Published 31 July 2020

ABSTRACT

Oil from *Allamanda cathartica* seed was extracted using n-hexane as solvent in soxhlet extractor. The physicochemical and antioxidants properties of the oil were carried out using standard analytical methods. The fatty acid attributes of the extracted seed oil were determined by Gas Liquid Chromatographic (GLC) method. The results of the physicochemical properties gave the followings: Specific gravity (0.87±0.04 g/cm^3), refractive index (1.46±0.02 at 30°C), viscosity (53.12±0.08 mpal/sec), acid value (69.17±0.12 mg/g), peroxide value (1.86±0.02 meg O_2/kg), saponification value (81.59±0.18 mg/g), iodine value (41.78±0.04 mg/100 g) and unsaponifiable matter (1.61±0.02%). The antioxidant analysis gave 2,2diphenyl picryl-1-hydrazyl radical scavenging capacity (DPPH) as (62.13±0.13%), ferric reducing antioxidant power (FRAP)

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(0.63±0.01%) and phenols (0.63±0.01%). The extracted oil was found to contain 64% saturated, 32% unsaturated and 4% polyunsaturated fatty acids. Margaric (23.74 g/100 g) and erucic acids (11.35 g/100 g) were the main saturated and unsaturated fatty acids, respectively in the seed oil. The overall results revealed that *Allamanda cathartica* seed oil is a potential source of plant oil alternative to the existing sources of oils.

Keywords: Allamanda cathartica; seed; oil; physicochemical; antioxidant; fatty acids

1. INTRODUCTION

Plants are very important in maintaining good health and quality of human life through numerous nutrients that abound in their leaves, stems, roots, fruits and seeds. Plant seeds are essential part of tropical agriculture since they are considered as source of nutrients for man and animals [1]. These nutrients, vitamins and mineral elements are useful for metabolic activities in the body. *Allamanda cathartica* is an evergreen climbing shrub that can reach a free climbing height of 2 meters or produce clambering stems of 6 meters or more that can grow into crowns of small trees. If not pruned, it can sprawl to a height of 20 ft. The plant is commonly called golden trumpet, yellow bell or butter cup flower. It is a widespread group throughout the world that belongs to the species of flowering plants, genus of *Allamanda* and Apocyanaceae family [2]. They are lower plants that include trees, shrubs and possibly vines. The plant is a perennial shrub or woody vine [3] while the plant seed capsules are oval and prickly. The leaves, roots and flowers are used in the preparation of cathartic while the milky sap is also known to possess antibacterial and possibly anticancer properties. The shrubs with their beautiful yellow flowers are popular ornamentals [4] and seed oils are important source of nutraceuticals. The characteristics of oils from different sources depend mainly on their composition. Biodiesel from unexploited plant seeds is fast becoming a promising alternative fuel for use in compression ignition diesel engines because of their low toxicity and high biodegradability. Oils from a single source cannot be suitable for all purposes; therefore individual study of oils from different sources for their constituents is important in order to be sure of its composition.

Around the world, there has been an increasing demand for oils from non-conventional sources to compliment the available ones [5]. Fatty acids are long hydrocarbon chains with a methyl group at one end and an acid group at the other. Unsaturated fatty acids are hydrocarbon chains containing at least one carbon-carbon double bond. Monounsaturated fatty acids contain one double bond while polyunsaturated fatty acids contain many double bonds. Fatty acids are known as important nutrients in both human and animal diets and also possess various health benefits [6]. Awika et al. [7] reported that it is necessary to boost the consumption of healthy foods through increase in the variety of food rich in beneficial compounds as well as improved processing approaches that enhanced their organoleptic usefulness.

Antioxidants are chemical compounds or mixture of compounds which when present in low concentration are used to prevent the oxidation of lipids, sugars and protein and DNA that can generate aldehydes, ketones, esters and other products that can be harmful to living systems [8]. Natural antioxidants have received considerable recognition in recent time due to the toxic and hazardous effects of synthetic antioxidants on human health. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive process caused by oxidative stress [9,10] and in decreasing the levels of dangerous body reactions that are produced by free radicals. These free radicals are associated with the ability to induce nutrition and medicine deterioration. In aerobic systems, there are reactive oxygen species (ROS) that reacts with the free radicals such as hydrogen peroxides, singlet oxygen, superoxide radicals, hydroxyl radicals and non free radical species [8]. Free radicals which are delivered as a consequence of typical biochemical responses in the body are involved in cancer, ischemic, heart disease, inflammation, diabetes, aging, atherosclerosis, immune suppression and neurodegenerative disorder [11].

The use of common synthetic antioxidants such as butylated hydroxyanisole (BHA) has declined due to suspected action as promoter of carcinogenesis as well as general rejection of

synthetic food additives [12] which hitherto has made the level of use to be under strict regulation [13]. Zheleva-Dimitrova and Balabanova [14] reported that oxidative damage caused by ROS has been associated with pathogenesis of various conditions in the body.

The need to explore plant materials to solve the problems of nutrition, health and industrial raw materials led to the choice of *Allamanda cathartica* seed - a less exploited seed for its oil characteristics as a means of expanding the scope of plant oils to be classified as alternative to the existing ones.

2. MATERIALS AND METHODS

2.1 Sample Collection and Treatment

The seeds used for this research work were collected from a local farm in Inisa, Odo -Otin Local Government area of Osun State, Nigeria. They were identified as *Allamanda cathartica* at the Biology Laboratory of Osun State Polytechnic, Iree, Nigeria by Mr. Akinro, Ebenezer Babatope with a specimen number of 0120 and placed in the Laboratory for reference. The seeds were sundried for two weeks after which the seed coat was removed. The dehulled seeds were further sundried for a week, pulverized using a laboratory blender, passed through a 2 mm sieve, packed in a polythene bag and kept in a dessicator ready for further analysis.

2.2 Methods

The oil was extracted from the seed flour in a soxhlet apparatus using n-hexane as solvent according to the procedure of George et al. [15]. The physicochemical properties of the extracted oil for specific gravity and refractive index were determined in line with the methods described by AOAC [16] while the other physicochemical properties were determined by the methods described by Tsado et al. [5]. The crude oil was methylated using the method describe by Lieb et al. [17] while the fatty acid methyl esters were analyzed following the method described by Ardabili et al. [18]. The antioxidant activity for phenolic content was measured with Folin-Ciocalteu reagent as described by Yu et al. [19], the free radical scavenging activity measured by 2,2 Diphenyl picryl-1-hydrazyl (DPPH) was done using the method highlighted by Shivani et al. [20] while the FRAP was analyzed using the method of Hinneburg et al. [21].

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 showed the physicochemical properties of the *Allamanda cathartica* seed oil. The specific gravity of 0.87 ± 0.04 g/cm^3 obtained for the oil was found to be similar to 0.85 g/cm reported for ackee pod oil [22] and 0.96 ± 0.01 for *Baphia sapida* seed oil [5]. The value was found to fall within the range of 0.87 – 0.90 g/cm recommended for biodiesel production by Duduyemi et al. [23]. Refractive index which is a measure of thickness and clarity of oils was 1.46 ± 0.02 at 30°C. This was similar to 1.45, 1.46 and 1.48 reported for the extracted oils from the aril, seed and pod of *B. sapida* [5] and extracted watermelon seed oil [23]. The sample seed oil is considered a non-drying oil as its refractive index value was not within the range of drying oils. Viscosity value of 53.12 ± 0.08 mPa.sec obtained for the oil was considered lower than 55.40 mPa.sec obtained for cashew nut seed oil [24]. Viscosity is an important parameter for consideration in industrial design to evaluate the quality of fats and oils in frying process [25]. The acid value of 47.36 mg/g is low compared to 77 mg/g documented for bread fruit seed oil by Bwai et al. [26] but higher than 0.78 ± 0.02 mg/g and 2.37 mg/g obtained for pumpkin [18] and watermelon [23] seed oils respectively. Acid value measures the extent to which glycerides in the oil have been decomposed by lipase and other physical factors [27]. The high acid value makes the oil unfit for cooking in the present form.

The peroxide value of the seed oil was found to be 4.20 ± 0.02 mg/kg. This value is relatively low compared to 9 meq O_2/kg and 12 meg O_2/kg reported for black and white seeds of watermelon grown in Sudan [28] and 10.85 ± 0.62 meq O_2/kg obtained for pumpkin seed oil by Ardabili et al. [18]. Despite the fact that the studied oil was unrefined, its peroxide value was within the range stipulated for freshly prepared oils which indicates that the oil has low concentration of trace elements and moisture and will therefore be more stable against rancidity. Saponification value of 262.30 ± 0.18 mg/g obtained for the oil was higher than 97.94 ± 0.24 mg KOH/g and 190.69 ± 1.40 mg/g reported for Olive oil by Borchani et al. [29] and pumpkin seed oil by Ardabili et al. [18] respectively. The oil would therefore be a useful and cheap raw material for soap production.

Table 1. Physicochemical properties of the seed oil

Parameter	Value ± S.D
Specific gravity (g/cm^3)	0.87 ± 0.04
Refractive index (at 30°C)	1.46 ± 0.02
Viscosity (mPa. sec)	53.12 ± 0.08
Acid value (mg/g)	47.36 ± 0.12
Peroxide value (meg O_2 /kg	4.20 ± 0.13
Saponification value (mg/g)	262.30 ± 0.20
Iodine value (mg/100 g)	41.78 ± 0.18
Unsaponifiable matter (%)	1.61 ± 0.02

n=3

Table 2. Antioxidants composition of the seed oil

Parameter	Value ± S.D
DPPH (%)	62.13 ± 0.13
FRAP (mg % ascorbic acid equivalent)	0.63 ± 0.01
Phenols (mg GAE/g)	0.63 ± 0.01

n=3

The iodine value of 41.78 ± 0.04 mg/100 g was lower than 116 ± 0.66 mg I_2/100 g reported for *B. sapida* seed oil by Tsado et al. [5] and 61.00 mg I_2/100 g reported for *Butrospermum parkii* by Aremu et al. [30]. The low iodine value signifies low degree of unsaturation, more vulnerable to oxidation, lesser ability to become rancid easily and unsuitability of the oil for paint making. This is in support of our submission on refractive index pointing to the nature of the oil. The iodine value fell outside the range of 220 -270 mg I_2/100 g specified for drying oils. The value of unsaponifiable matter of 1.61% obtained for *Allamanda cathartica* seed oil was within the range of 1.2 - 1.8% reported for black cumin seed oils [31] and also comparable to the values obtained for common vegetable oils by Karleskind [32].

Table 2 showed the antioxidants composition of the seed oil. The DPPH value was found to be 62.13 ± 0.13%. This value was higher than 29.92% and 19.94% reported for *Jatropha curcas* leaf extract and seed oil respectively [20]. The DPPH result revealed the usefulness of the seed oil as a source of natural antioxidant. The oil has a FRAP value of 0.63 ± 0.01 mg % ascorbic acid equivalent and this is higher than 0.49 and 0.27 mg % ascorbic acid equivalent reported for the aqueous and hexane fractions of *Thaumatococcus danielli* leaves [33]. Phenol value of 0.63 ± 0.01 mg GAE/g was considered lower than 125.44 ± 0.182 mg GAE/g and 129.97 ± 0.087 mg GAE/g obtained for ethanolic extracts of *Convolvulus austroaegytiacus* and *Convolvulus pilosellifolius* by Al-Ritai et al. [11] and also lower than 0.96-7.60 mg GAE/g reported by Haron et al. [31] for some oils from Yemen, Iran and Malaysia. Phenolic content is considered an oil quality assessor in that it is related to colour, shelf life as well as resistance to oxidation [29]. It has been documented that phenolics showed high levels of antioxidant activity [34] and contributes to the stability of the oil under accelerated oxidation conditions [31].

Table 3 reported the fatty acid composition of the seed oil. The oil contains 64% saturated fatty acids, 32% unsaturated fatty acids and 4% polyunsaturated fatty acids. Among the saturated acids determined, margaric acid was found to be major fatty acid constituents of the seed oil with 23.75 g/100 g. It was followed by stearic acid (12.78 g/100 g) while lauric acid gave the least value. The presence of margaric acid in food materials helps to reverse the early stage of diabetes. Palmitic acid of 7.98 g/100 g is low compared to 10.64% and 25.76% reported for *Citrullus vulgaris* seed oil and palm kernel oil by Raul and Shrinvas [35] and Atasie and Akinhanmi [36] respectively. Stearic acid content of 12.78 g/100 g in the seed oil was considered to be higher than 0.64% reported for palm kernel oil [36].

A total of 11 fatty acids were detected in the seed oil and the sum of the values obtained for the fatty acids was 81.00 g/100 g oil, this is similar to 79.34 and 87.578 g/100 g oil obtained for apple and pear seed oils by Rui et al. [37]. The presence of minor fatty acids in the unrefined oil accounted for the remaining 19.00 g/100 g

reported as others (Table 3). Behenic acid was found to be 1.34 g/100 g. This value was higher than the range of 0.25-0.32 g/100 g obtained by Sonwai and Ponprachanuvut, [38]. The presence of behenic acid would make the seed oil useful as hair conditioner and moisturizer. The quantity of the major unsaturated fatty acids determined were oleic acid (14.57 g/100 g) and erucic acid (11.35 g/100 g). The value obtained for oleic acid was low compared to the range of values obtained by Sonwai and Ponprachanuvut [38] for fats from various Thai varieties of mango kernels.

Table 3. Fatty acid composition of the seed oil (g/100 g)

Fatty acid	Value ± S.D
Lauric acid C12:0	0.83 ± 0.01
Myristic acid C14:0	1.55 ± 0.02
Pentadecanoic acid C15:0	1.88 ± 0.01
Palmitic acid C16:0	7.98 ± 0.10
Margaric acid C17:0	23.75 ± 0.05
Stearic acid C18:0	12.78 ± 0.50
Arachidic acid C20:0	2.02 ± 0.01
Behenic acid C22:0	1.34 ± 0.02
Oleic acid C18:1	14.57 ± 0.50
Erucic acid C22:1	11.35 ± 0.02
Eicosadienoic acid C20:2	2.95 ± 0.01
Others*	19.00

n=3

Summary of the amino acid compositions (g/100 g) and (%)

Saturated fatty acids (SFA) = 52.13 (64 %)
Unsaturated fatty acids (UFA) = 25.92 (32 %)
Polyunsaturated fatty acids (PSFA) = 2.95 (4 %)
*Represent other minor amino acids = 19.00

Oleic acid in foods acts as inflammatory mediator as well as for promotion of antioxidant defense. Erucic acid is an important raw material in production of detergents, inks and lubricants. It may be subjected to several processes to produce erucamide which is used as slip agent in plastic films. Eicosadienoic acid content of 2.95 g/100 g was the only polyunsaturated acid determined in the seed oil. Polyunsaturated fatty acids help to reduce cholesterol levels in the blood and indirectly lower the risk of heart disease. The total sum of saturated fatty acids gave the highest value of 52.13 g/100 g (64%) compared to 25.92 g/100 g (32%) and 2.95 g/100 g (4%) recorded respectively for the total sum of unsaturated and polyunsaturated fatty acids. The trend of saturated fatty acids > unsaturated fatty acids > polyunsaturated fatty acids obtained was similar to the findings of Bwai et al. [26] for breadfruit.

4. CONCLUSION

The *Allamanda cathartica* seed oil generally was found to have better quality of antioxidants, fatty acid profiles and physicochemical properties that may be adapted as cheap source of raw material to provide more wide and novel applications in agro-based, cosmetics and pharmaceutical industries. The polyphenols and the antioxidants characteristics of the seed oil can also be utilized in products that require stability.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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