

Original Research Article

Physicochemical study, fatty acid profile and antioxidant potential of the seed oil of *Polyalthia longifolia angustifolia*

ABSTRACT

In the face of worsening malnutrition or undernutrition in poor countries, diversification of food resources remains a high priority for the World Health Organisation (WHO). Thus, the present study aims to determine the phytochemical composition and antioxidant activity of the fat extracted from the seeds of *Polyalthia longifolia angustifolia*.

The fatty acid profile of the oil, extracted from the seeds with hexane by the Soxhlet method, was determined by gas chromatography-mass spectrometry (GC/MS) analysis. The physicochemical parameters of the oil were measured according to AFNOR standards. The total phenol and flavonoid contents of the seeds were evaluated by UV-visible spectrophotometric measurement. Antioxidant activity of the oil was determined by the reduction test of ferric ions (Fe^{3+}) of the TPTZ complex to ferrous ion (Fe^{2+}). The results revealed that the oil contains a higher level of oleic acid (63.38%), followed by: palmitic acid (13.80%); linoleic acid (11.74%) and stearic acid (6.24%). The physicochemical parameters of the oil were: refractive index (1.499); acid value (87.69 mg KOH/g of oil); acidity (23.659 g oleic acid/100 g of oil); iodine value (78.26 g I_2 /100 g of oil); saponification value (179.741 mg KOH/g of oil) and peroxide number (10.85 meq O_2 /kg of oil).

In short, the seeds of *P. l. angustifolia* contain fat and phytochemicals with appreciable antioxidant activity. However, further studies are needed to evaluate the toxicity, nutritional and therapeutic properties of the oil before considering the different types of applications that can be made with this oil.

Keywords: *Polyalthia longifolia angustifolia*, seeds, oil, nutritional value.

1. INTRODUCTION

The benefits of vegetable oils have been known for a very long time. They are used in various fields, including food processing, transport, medicine and cosmetics. In nutrition, vegetable oils are an essential part of the human diet [1]. Indeed, they are the most important source of energy for humans and provide essential fatty acids such as linoleic acid and alpha-linolenic acid, which are necessary for the proper functioning of the body and the maintenance of its nutritional balance [2]. In most poor countries, usually exposed to food insecurity, diversification of food resources remains one of the main priorities for the WHO. In this context, explorative research on oilseeds continues to be a necessary strategy to reduce hunger in the world. *P. l. angustifolia* is a plant that has its geographical origins in

India and Sri Lanka. It has been domesticated in other areas, in particular in Asia and even in West Africa, Central Africa, East Africa and Madagascar. In general, it is grown for use as an ornamental tree, sun protection (shadow) and noise pollution control [3]. Some of its organs, such as bark, stem and leaves, have been the subject of various medicinal or phytosanitary uses [4]. Thus, many previous studies have been conducted in literature on the bark and leaves of the plant [5]. However, few studies have focused on the roots of this plant. Moreover, to our knowledge, its seeds have not yet been studied for phytochemical screening, nor have its fruits, which are not yet consumed by humans. They are therefore considered as waste and only serve as food for bats. Although it is not yet known as an oil plant, perhaps because of the low fat content of its seeds, *P. l. angustifolia* is a plant whose vegetable oil extracted from the seeds could be full of molecules with useful properties for humans.

The present study focused on the valorization of *P. l. angustifolia* seeds in the food chain through the determination of the phytochemical composition and the antioxidant activity of their fat.

2. MATERIAL AND METHODS

2.1. Plant material

The seeds of *P. l. angustifolia* were harvested during May 2019 in Agoé, located in Golfe department, in the south of Togo. After three weeks of drying in the dark and at room temperature (28-30°C), the seeds were manually dehulled, crushed and then ground. The resulting powder was put into a plastic bottle, closed and stored in the freezer for later use.

2.2. Phytochemical quality screening of seeds

Staining and/or precipitation reactions were used to test for the presence of phytoconstituents such as: alkaloids, flavonoids, tannins, saponosides, reducing compounds, and carbohydrates [6,7,8].

2.3. Extraction of crude vegetable oil from seeds

Hexane was used as a solvent to extract crude vegetable oil (CVO) by applying the Soxhlet method from the seeds of *P. l. angustifolia*.

2.4. Determination of physicochemical parameters of the CVO

The refractive index, acid number, acidity, saponification number, iodine number and peroxide number are the physicochemical parameters that were determined according to AFNOR (1984) [9] standards for CVO of our *P. l. angustifolia* seeds.

Two trials were carried out for the measurement of each parameter and the result was expressed as the calculated mean \pm standard error.

2.5. Analysis of the fatty acid profile of the CVO

2.5.1. Transmethylation of the CVO for GC-MS analysis

In a haemolysis tube fitted with a screw cap, 20 mg of CVO was solubilised in 5 mL of a methanolic solution of sulphuric acid (2.5%). The mixture was homogenised in small bulb, sealed and then heated for 90 min in an oven set at 80°C. After heating, a volume of 1.5 mL of a sodium chloride solution NaCl (0.9%) was added to the mixture. Then, after vigorous stirring, a volume of 1.5 mL of hexane was added to the final solution to extract the methyl esters of the fatty acids which were formed.

2.5.2. GC-MS analysis of prepared fatty acid methyl esters

The previously prepared sample of fatty acid methyl esters was analysed by gas chromatography-mass spectrometry (GC-MS) system. The analysis was performed using a TRACE 1300 Series GC chromatograph, equipped with a DB5-MS capillary column (length: 50 m; inner diameter: 0.25 mm and film thickness: 0.25 μ m), fitted with an AIS/AS 1310 Autosampler and coupled to an ISQ MS Tune mass spectrograph, fitted with an electron

impact detector. XCalibur software was used for data acquisition. The temperature of the mass spectrograph was set at 250°C. The spectra were recorded with 70 eV. The conditions for the analyses are set as reported by Novidzro et al. (2019) [10]. The analytical conditions were set as follows: injector temperature: 250 °C; oven temperature

2.6. Quantitative analysis of total flavonoids and polyphenols of the CVO

Total polyphenols were determined using the method described by Singleton et al. (1999) [11], based on the use of the Folin Ciocalteu reagent (FCR). Gallic acid was used as the standard to plot the calibration curve (Figure 1 A). Total flavonoids were quantified according to the method described by Zhishen et al. (1999) [12]. Quercetin was used as a reference to establish the calibration curve (Figure 1 B).

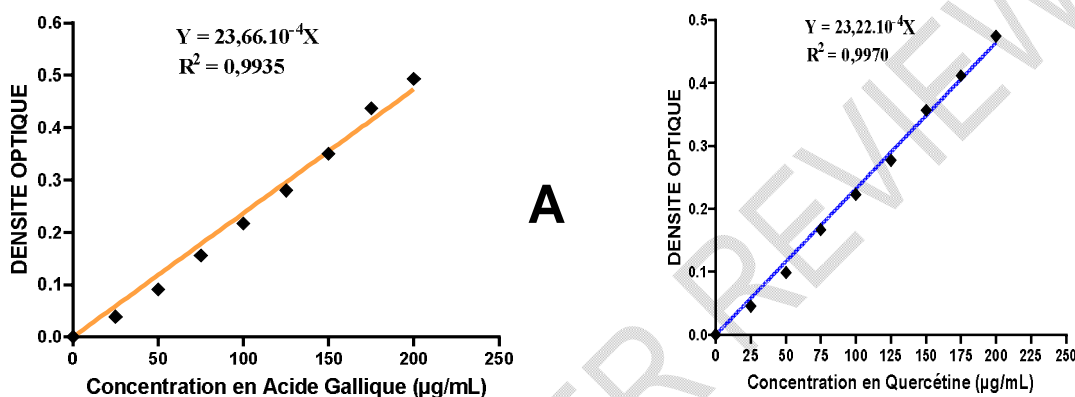


Figure 1: Calibration curves for gallic acid (A) and quercetin (B)

2.7. " In vitro " evaluation of antioxidant capacity of the VCO

The FRAP test for the reduction of the ferric tripyridyltriazine complex $[(Fe(III)TPTZ)_2]^{3+}$ to the ferrous tripyridyltriazine complex $[(Fe(II)TPTZ)_2]^{2+}$ was carried out to evaluate the antioxidant capacity of *P. l. angustifolia* seed oil by the UV-Visible spectrophotometric method following the experimental approach described by Nair et al. (2007) [13].

The absorbance reading was measured at a wavelength of 593 nm using a METASH UV-Visible spectrophotometer (UV-5200 PC). Data acquisition was done with the MetaSpec Pro software, against the blank, after 10 min of incubation. A calibration curve (Figure 2) was established with a solution of iron sulphate ($FeSO_4 \cdot 7H_2O$), dissolved in methanol. The result was expressed as $\mu mol Eq FeSO_4/mg$ of oil.

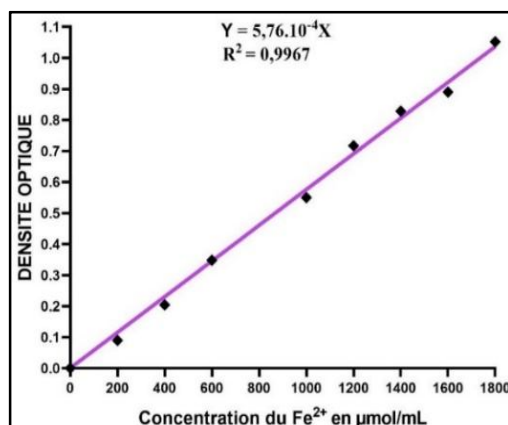


Figure 2: Calibration curves for iron sulphate (FeSO₄, 7H₂O)

2.8. Statistical analysis of the data

The data collected in this study were recorded in Excel 2010 and processed using GraphPad Prism software, version 8.4.3. Differences between the various values measured were considered significant at the 5% accuracy level. The result of each measured value was presented as the mean \pm standard error of the mean.

3. RESULTS AND DISCUSSION

3.1. Phytoconstituents identified in seeds

Phytochemical screening qualitatively performed on *P. l. angustifolia* seeds revealed the presence of phytochemicals (Table 1), such as: alkaloids, flavonoids, tannins, saponosides, phenolic compounds, reducing compounds, cardiac glycosides and carbohydrates.

Table 1: Phytochemical compounds revealed in the seeds of *P. l. angustifolia*

Phytochemical components	Seeds of <i>P. l. angustifolia</i>
Alkaloids	+
Flavonoids	+
Tannins	+
Saponosides	+
phenolic compounds	+
Reducing compounds	+
Cardiac glycosides	+
carbohydrates	+

Legend: + = presence

The seeds of *P. l. angustifolia* contain various phytochemical groups such as: alkaloids, flavonoids, tannins, saponosides and carbohydrates (Table 1). The presence of these phytochemical constituents shows that *P. l. angustifolia* seeds constitute a source of biomolecules whose characterisation could guide their use for therapeutic and other purposes.

The presence of various secondary metabolites in the seeds then justifies the use of this plant as a traditional remedy for some ailments [4]. Indeed, the bark of the trunk has been used to treat diabetes and later to lower blood pressure and fever [3]. In India, the plant is used in traditional medicine as a febrifuge and tonic [14,15].

3.2. Physicochemical characteristics of the CVO

The results of the measurements of the physicochemical parameters of the COV of *P. l. angustifolia* are presented in Table 2.

Table 2: Quality indexes of the CVO of *P. l. angustifolia* seeds

Physicochemical parameters determined	Results
Fat content (%)	5.00 ± 0.012
Refraction index	1.499 ± 0.001
Acid value (mg KOH/g oil)	87.69 ± 0.53
Acidity (g oleic acid/100 g of oil)	23.66 ± 0.36
Saponification value (mg KOH/g of oil)	179.74 ± 0.68
Iodine value (g I ₂ /100 g of oil)	78.26 ± 0.15
Peroxide number (µg O ₂ /kg of oil)	10.85 ± 1.55

The fat content of *P. l. angustifolia* seeds was estimated at 5.00% showing that the oil content of the seeds is relatively very low (Table 2).

The refractive index value of the oil, 1.498, is higher than that specified by the Codex Alimentarius standards for food fats and oils (1.463-1.478) [9]. This indicates that the oil is not suitable for food consumption in its crude state. However, it is in the range of the refractive index of the oils known as siccatives (1.480-1.523) and can therefore be classified as a stable oil, which is highly valued by the paint industry [9].

The oil has a relatively high acid value, 87.69 mg KOH/g of oil. This value is much higher than those recommended for fats and edible oils, i.e. between 0.6 and 4.0 mg KOH/g of oil [16]. This implies that this oil contains a fairly high amount of free fatty acids (FFAs). The existence of these FFAs in relatively large quantities is prejudicial to its preservation because the oil is predisposed to a greater ease of rancidity. The high free fatty acids (FFAs) content could be due to the ripeness of the seeds used for extraction or to a hydrolysis reaction of glycerol esters that occurs during extraction (heat) and storage of the oil [17]. It has also been found that triacylglycerol lipases are enzymes capable of hydrolysing triglycerides containing long-chain fatty acids by giving off free fatty acids [18]. Furthermore, in the presence of water, the FFAs initially present in the reaction medium act as catalysts for the hydrolysis reaction that occurs between triglycerides and water by generating again, other FFAs in the extraction medium [19]. However, ester hydrolysis is a process that also develops mainly during storage and even in the absence of any enzymatic activity, in a water-saturated oil [19]. According to the Codex Alimentarius standards, an oil with more than 5% FFAs is considered unfit for consumption [9]. According to this criterion, this oil is not edible, as it has a very high FFAs content (23.66%).

It is recognised that the stability of an oil during storage depends on its initial characteristics and the conditions under which it is stored [1]. However, in order to avoid qualitative degradation of an oil, in particular rancidity, it is advisable to extract the oil quickly just after harvesting the seeds. Therefore, *P. l. angustifolia* oil requires a subsequent refining step to remove FFAs and impurities. However, during refining, precautions must be taken to avoid destroying the useful constituents such as carotenoids and vitamins [20] in order to preserve the nutritional values of the oil.

The iodine value of the oil, 78.26 ± 0.15g I₂/100g oil, suggests that it contains enough unsaturated fatty acids. This value is lower than that of the oil from *Polyalthia longifolia pendula* seeds, i.e. 95 g I₂/100 g of oil obtained by Oyedeji et al. (2011) [21]. However, it is relatively close to the iodine value of olive and peanut oils, i.e. 75 and 94 I₂/100 g of oil respectively [22]. Based on the relatively high iodine value of this oil, it therefore has a high

sensitivity to oxidative rancidity [23]. Therefore, it is wise to take the necessary precautions to ensure good stability for this oil during storage to reduce the risks associated with auto-oxidation reactions.

In this study, the saponification value of for *P. I. angustifolia* CVO is about 179.741 mg KOH/g oil (Table 2). This value is lower than the range notified by the Codex Alimentarius standard [9] for edible fats and oils, varying between 189 and 195.2 mg KOH/g of oil. The low value of this index can be explained by the high proportion of long carbon chain fatty acids in the oil. However, this value is not relatively far from that of olive oil, i.e. 187-196 mg KOH/g of oil [24]. On the other hand, it is much higher than that of *Polyalthia longifolia pendula* oil, i.e. 120 mg KOH/g of oil [21]. According to the saponification value of our oil, it could be classified as a fat for cosmetic applications.

The peroxide number of our CVO was $10.85 \pm 1.55 \mu\text{g O}_2/\text{kg}$ of oil (Table 2). This value is higher than that regulated by the standard for conventional oils [16], i.e. $10\text{-}15 \mu\text{g O}_2/\text{kg}$ of oil. The double bond in the carbon chain of a fatty acid is a vulnerable point and makes it susceptible to oxidation or peroxidation reactions [2]. These oxidation reactions are extremely complex and lead to the formation of different kinds of products.

3.3. Fatty acids found in the CVO of *P. I. angustifolia* seeds

The GC analysis provided the chromatogram shown in figure 3 and after MS detection, the results provided by the XCalibur software about the composition of the chemical fatty acids of *P. I. angustifolia* CVO are reported in Table 3.

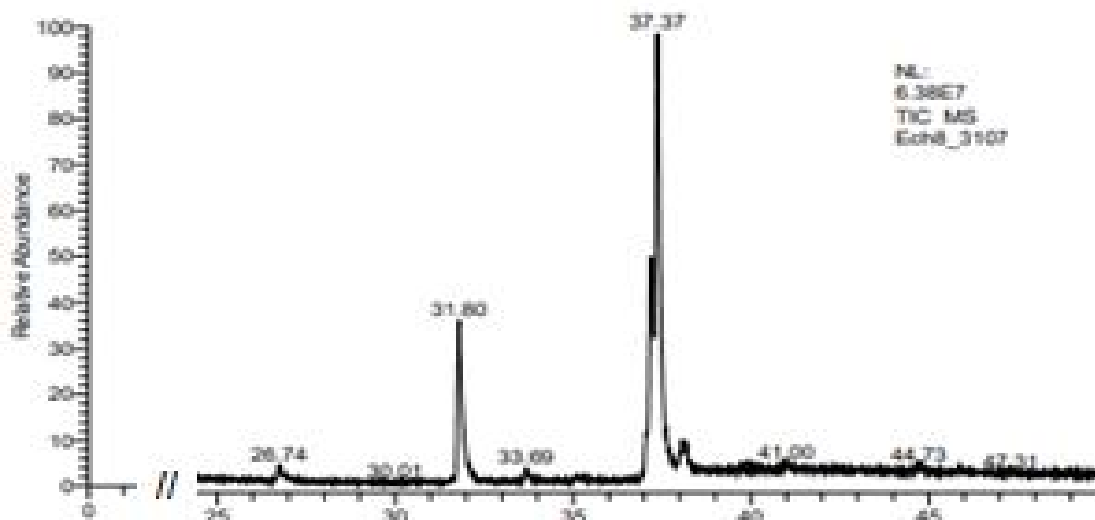


Figure 3: GC analysis chromatogram of CVO of *P. I. angustifolia* seeds

Table 3: Fatty acid composition of CVO of *P. I. angustifolia* seeds

Fatty acids detected in the CVO	Rates (%)
Myristic acid (C14 : 0)	0.02
Palmitic acid (C16 : 0)	13.80
Stearic acid (C18 : 0)	6.24
Oleic acid (C18 : 1 ω^9)	63.38
Linoleic acid (C18 : 2 ω^6)	11.74
Saturated fatty acids (SFA)	20.06
Unsaturated fatty acids (PUFAs)	74.12

This study reveals that *P. l. angustifolia* oil is an excellent source of unsaturated fatty acids (UFAs), predominantly composed of oleic acid (63.38%) and linoleic acid (11.74%). On the other hand, this oil contains 20.06% of saturated fatty acids (SFAs), of which palmitic acid is the most important, with a content of around 13.80%. The ratio of MUFAs to SFAs is 3.69, which shows that MUFAs are considerably more representative than SFAs. This oil is comparable to olive oil with an oleic acid content of 50-80% [25]. Oleic acid is the most abundant monounsaturated fatty acid and is an excellent source of energy for our bodies. Its role is to protect the cardiovascular system by lowering blood pressure. It also reduces bad cholesterol levels while increasing good cholesterol levels [26]. Because it cannot be synthesised by our body, Oleic acid has been recognised as an essential fatty acid to be supplied by the diet. Its main function is to lower cholesterol levels. In addition to, it has anti-carcinogenic properties, anti-atherosclerosis activity and reduces obesity [27]. The palmitic acid present in HVB plays a necessary role in the proper functioning of our body by combining with our cells (liver, pancreas, stomach) to improve their functioning. It is provided through energy intake from our diet or synthesis by our liver [28].

3.4. Total flavonoid & total phenol contents and antioxidant activity of the CVO

The contents of total flavonoids & total phenols and antioxidant capacity of *P. l. angustifolia* seed oil are shown in Table 4.

Table 4: Total phenol & flavonoid contents and antioxidant capacity of the CVO

Values assessed	Contents
Total Phenols	33.96 ± 0.04 mg FAME/g of oil
Total flavonoids	15.30 ± 0.31 mg Eq/g of oil
Antioxidant power	253,31 ± 0,66 µmol Eq FeSO ₄ /mg of oil.

Legend: FAME = Fatty acid methyl esters and Eq = equivalent

The contents of total phenols and flavonoids in the CVO extracted from *P. l. angustifolia* seeds are very low. This is because the method of oil extraction based on hexane, which is an apolar solvent, is not efficient to extract these biomolecules, since most of the phenolic compounds (phenols and total flavonoids) are polar molecules that have little affinity with apolar solvents. Nevertheless, according to the literature, there are some complex phenols called secoridoids with lipophilic properties that are found in some vegetable oils such as virgin olive oils obtained by mechanical pressing [29].

The presence of phenolic compounds should confer antioxidant activities to this oil, as the literature indicates that they are powerful antioxidants. Their role in the organoleptic quality of food is considered very important. If our CVO extracted from the seeds of *P. l. angustifolia* were to be consumable, then it would eventually provide the corresponding organism with protection of its antioxidant defence systems. The presence of some of the flavonoids in this oil could give it many biological activities as flavonoids are able to modulate the activity of many enzymes and modify the behaviour of several cellular systems. Indeed, it has been suggested that flavonoids may exert a multitude of biological activities including significant antioxidant, vasculoprotective, anti-hepatotoxic, anti-allergic, anti-inflammatory, anti-ulcer and even antitumor properties [30,31].

The evaluation of antioxidant activity based on FRAP test revealed that our oil has a rather interesting antioxidant activity, evaluated at 253.31 ± 0.66 µmol Eq FeSO₄/mg of oil. This antioxidant power could probably depend on the presence of phenolic compounds contained

in CVO. However, given the low content of total phenolic compounds, other minor constituents present in the oil including: carotenoids, vitamin E (tocopherols and tocotrienols), vitamin D and provitamin A, could also contribute to the antioxidant activity by synergistic action [1].

CONCLUSION

The present work allowed us to determine the phytochemical composition of *P. l. angustifolia* seeds and to assess the physicochemical properties, the fatty acid profile and the antioxidant power of its CVO. Overall, the values of the various physicochemical parameters measured showed that this oil is not suitable for human food consumption. Indeed, the values of the acid, iodine and peroxide number show that the oil is very sensitive to oxidation and therefore, necessary precautions must be taken during its conservation in order to preserve its initial characteristics.

Owing to its high acidity, this oil cannot be used as a food oil without prior treatment. As the oil has a rather interesting antioxidant capacity, its use in other applications may be beneficial for humans.

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