Fatty Acid Profiles, Antioxidant and Phenolic Contents of Oils Extracted from *Acacia polycantha* and *Azadirachta indica* (Neem) Seeds using Green Solvents

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Abstract

The purpose of the present study was to evaluate the composition of fatty acids, antioxidant and phenolic compounds in *Acacia polycantha* and *Azadirachta indica* seed extracts for their potential uses in nutrition. Extractions of oil using several techniques (Sohxlet, ultrasound and microwave) in several solvents were carried out and the oils were characterised. Total phenolic content ranged between 4.22 and 31.48 mg GAE/g. Antioxidant activity (CE_{50}) ranged between 1.95 and 22.91 mg/mL. Oleic and linoleic acids were the major unsaturated fatty acids while palmitic and stearic acids were the major saturated fatty acids in both oils. 3-hydrobenzoic acid, resveratrol rutin, and flavan were identified by HPLC-DAD at high contents while chlorogenic acid, vanillic acid, syringic acid, epicatechin, *p*-coumaric acid, transferulic acid, ellagic acid, rutin, cinnamic acid, chlorogenic acid, caffeic acid were lower in Neem oil. In case of *A. polycantha* oil, the percentages of ellagic acid and rutin were high.

Keywords: *Acacia polycantha*, *Azadirachta indica*, seeds, fatty acids, antioxidant, phenolic compounds

Practical applications

This study investigated the best method which gives a high oil extraction yield from among Sohxlet, ultrasound and microwave using green solvents. Results revealed that acetone and isopropanol can be used for oil extraction from seeds using microwave or ultrasound with shorter extraction times (30 minutes with ultrasound and 15 minutes for microwave). These solvents provided oils with high phenolic content and good antioxidant properties. The main fatty acids identified were oleic, linoleic, palmitic and stearic acids. These oils thus have a high potential for pharmaceutical, food and cosmetic industrial uses.

1. Introduction

The demand for edible oils and biofuel is steadily growing in the international market. This explains why several studies have been conducted to characterize oils from fruits, seeds and barks of trees (Youzbachi *et al*., 2015). These oils are used for the treatment of several diseases in sub-Saharan Africa where more than 80% of the population depends on traditional medicine (Rao *et al*., 2019). Conventional Soxhlet extraction of oil consumes a lot of energy. In recent decades, researchers have

developed new methods that consume less energy and use green solvents that are more protective of the environment (Castejón *et al*., 2018). Recommended alternative solvents to hexane in the food industry for the extraction of oils are ethanol, water, acetone, isopropanol, *n*-butanol, ethyl acetate… (Castejón *et al*., 2018; Prat *et al*., 2016).

Pressurized fluid extraction, microwave-assisted extraction, ultrasound-assisted extraction, enzymatic extraction, pulsed electric field extraction, and supercritical fluid extraction are new techniques that have been tested as alternatives to conventional solid-liquid extraction. Many researchers have shown that the ultrasound method greatly reduces extraction times, can be easily reproduced, can improve extraction yields and can extract many more compounds (Goldsmith *et al*., 2018; Perrier *et al*., 2017, Liu *et al*., 2015). Microwave-assisted extraction can be considered a green extraction technique allowing for a significant reduction in extraction times, better extraction yields, smaller amounts of solvent and therefore lower costs. This method has been used for extracting bioactive compounds (Isopencu *et al*., 2019; Llompart *et al*., 2019).

Neem (*Azadirachta indica*), a tropical tree native to India, is an excellent wind break and all parts of the tree are useful. The roots, leaves, bark, seeds and flowers of the Neem have been used for many years in traditional medicine to treat many diseases and disorders (Biswas *et al*., 2002; Rao *et al*., 2019). Neem oil, extracted from the seeds, is used as a raw material for the production of pesticides and soap, for the protection of agricultural stock and textiles, as lubricant for engines and for the treatment of several diseases such as diabetes and tuberculosis (Acharya et al., 2017; Orhevba et al., 2013). In agriculture, Neem leaves, crushed and soaked in water, have been used as a fungicide and insecticide for the treatment of plants pathogens (Adewoye and Ogunleye, 2012; Liauw *et al*., 2008; Nde *et al*., 2015). Research has shown that Neem extracts have bioactive molecules with anti-cancer, anti-malarial (Iwu *et al*., 1986), insecticidal (Isman *et al*., 1990; Lee *et al*., 1991), larvicidal and spermicidal (Riar *et al*., 1990), anti-bacterial, anti-fungal, anti-viral, anti-inflammatory (Akhila and Rani, 1999) properties. Azadirachtins are important active ingredients contained in the Neem seed. Several active compounds such as salannin, genudin, nimbin, quercetin, nimbinin, nimbidin, (Ambrosino *et al*., 1999; Fernandes *et al*., 2019; Rahmani *et al*., 2018) have been isolated. Research has highlighted the presence of many fatty acids in Neem oils. These oils are generally richer in unsaturated fatty acids than in saturated fatty acids (Diedhiou 2017; Faye 2010). Despite the extensive literature on Neem, the seeds from Cameroon have not been studied.

Acacia polyacantha (*Acacia campylacantha*) belongs to the family of Leguminocae – Mimosoideae and is found in tropical Africa. Gastro-intestinal infections and livestock diseases are treated traditionally using this plant. It is also used as an antidote against veminomous sting. The roots and bark are used to treat diarrhoea, dysentery, naso-pharyngeal affections, stomach troubles, veneral diseases, etc (Burkill 1985; Fotso *et al*., 2018; Koudoro *et al*., 2015; Mambe *et al*., 2019). Research has shown that *Acacia polyacantha* extracts contain bioactive molecules that have antibacterial, anti-helmintic properties (Mambe *et al*., 2019; Waterman *et al*., 2010). Several compounds have been isolated from the leaves and bark of this plant (Fotso *et al*., 2018; Mambe *et al*., 2019). However, in Cameroon, no study has been carried out yet on the characterization of *Acacia polyacantha* seeds and on the the antioxidant activity and phenolic content of Neem oil extracted from seeds. Therefore, the objective of the present investigation was to evaluate the suitability of ultrasound- and microwave-assisted extraction as compared to the Soxhlet technique for the recovery of oil from *A. polycantha* and from Neem seeds. The best green solvent from among acetone, isopropanol and ethanol as compared to hexane solvent for oil extraction was also identified. Finally, the effects of various oil extraction techniques were evaluated on the basis of oil yield, fatty acid composition, phenolic content, antioxidant content and phenolic composition.

2. Materials and methods

2.1 Reagents

n-Hexane (Biosolve Chimie Sarl, HPLC, 95%), isopropanol (Scharlau), ethanol (96%), acetone (Scharlau), distilled water, methanol (Scharlau, > 99.8%), sodium carbonate (Jansen Chimica), gallic

acid (Sigma Aldrich, 97.5-102.5% titration), Folin-Ciocalteu reagent (Sigma Aldrich), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich), 2,6-di-tert.butyl-4-méthyl-phénol [BHT] (> 99%, Fluka), FAME 37 (Supelco) and diethyl ether (Scharlau, 99.5%) of reagent grade, were used without further purification.

2.2 Raw materials

For this study, *A. polycantha* and Neem seeds were obtained from the Far North region, Diamare division, and identified by a specialist of the "Herbier National du Cameroun" in Yaoundé. After harvest, whole seed and hull were air-dried. The seeds were then recovered by dehulling and the hulls were kept for other uses. The seeds were ground using a cutter mill (Retsch Gmbh type SR2) to a particle size less than 0.5 mm. The powder obtained was kept in the dark for extraction and other analyses.

2.3 Extraction of oil

Three types of extraction, Soxhlet, ultrasound and microwave, were performed. Five solvents: *n*-hexane, isopropanol, ethanol, water and acetone were used.

2.3.1 Soxhlet extraction

The extraction was carried out on 6 g of powdered seed in 60 mL of solvent (*n*-hexane, isopropanol, ethanol, or acetone) in a 100-mL Soxhlet extractor for 18 h at the rate of 7 siphons per hour. This time was chosen to ensure maximum extraction. Then, the solvent was evaporated under reduced pressure at 40°C using a BUCHI rotavapor (R-114) and a water bath BUCHI (B-491), vacuum controller BUCHI (V-850) and the crude oil was dried under vacuum in a desiccator over phosphorus pentachloride, P_2Cl_5. The oil was weighed and stored in the dark for further use (Saha *et al*., 2018). The yield of oil content was calculated using equation 1.

$$\text{Oil yield}(\%) = \frac{m_1}{m_2} \times 100 \quad (1)$$

where m_1 is the mass of the oil extracted, m_2 is the mass of oven-dried sample. All the analyses were conducted in triplicate.

2.3.2 Ultrasonic extraction

Extraction was carried out using a VWR Ultrasonic Cleaner USC 300D (Malaysia), with a frequency of 45 kHz - 80 W, a generator of 230 V/50-60 Hz, 300 VA with a 160 W heating capacity. About 7 g of material was introduced into a vial and 70 mL of solvent was added (*n*-hexane, isopropanol, ethanol, or acetone). The mixture was well shaken and placed in the extractor at the temperature of 50°C for 30 minutes. The mixture was filtered on filter paper n° 1 under vacuum, the solvent removed by rotary evaporator, the oil dried and weighed. The oil extraction yield was calculated using equation 1.

2.3.3 Extraction assisted by microwave heating

In an extraction tube, 6 g of material was mixed with 60 mL of solvent (*n*-hexane, isopropanol, ethanol or acetone) and extracted for 15 minutes using a Milestone Start SYNTH type microwave at 200 W power at a temperature of 50°C. This total time of treatment, 15 min, was for temperature rise, extraction time of 5 min and cooling of microwave. It should be noted that a longer time of extraction at high temperature can lead to a calcination of the sample. After extraction, the mixture was filtered under vacuum and the solvent removed by rotary evaporator and the oil dried and weighed. The yield of oil extracted was calculated as previously described.

2.4 Determination of total phenolic content

The total phenolic content of oil was evaluated by the method described by Folin-Ciocalteau (Chiara *et al*., 2018). To 40 g of oil was added 3160 µL of MeOH/H_2O (50:50, v/v) solution. The mixture was shaken and 200 µL of Folin-Ciocalteau reagent, diluted 10 times in water, was added. The mixture

was left in the dark for 8 minutes and then, 600 µL of aqueous solution of sodium carbonate (20%, w/v) was added. The mixture was then stirred and incubated for 2 hours in the dark at room temperature. After incubation, the mixture was centrifuged at 5000 rpm in a Beckman Coulter Centrifuge for 5 minutes and the absorbance of the supernatant fluid was read at 765 nm using a UV-Visible spectrophotometer (UV-1800 Shimadzu). The total phenolic content was expressed as mg of gallic acid equivalent per g of oil read from the calibration curve of gallic acid standard solutions. Equation 2 was used to calculate the total phenolic content (TPC) (Alara *et al*., 2017).

$$TPC\ (mg\ GAE/g) = \frac{\mathrm{C\ x\ V}}{m} \qquad (2)$$

where C is the concentration read from the calibration curve (mg/mL), V is the volume of the solvent (mL) used for the extraction, and m is the mass of the oven-dry sample used (g).

2.5 Determination of antioxidant activity

Antioxidant activity of oil was determined by the method described by Zhong *et al*. (2018) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as radical scavenger.To 0.2 mL of oil was added 10 mL of methanol and the mixture was shaken vigorously for 1 minute and vortexed for 30 seconds. The mixture was centrifuged at 3000 rpm for 5 min. 0.6 mL of supernatant solution was added to 2 mL of DPPH solution (0.1 mmol/L in methanol). The mixture was incubated in the dark for 1 hour and its absorbance was read at 517 nm using the UV-Visible spectrophotometer. The antioxidant activity was calculated by the efficient concentration CE_{50}, which is the antioxidant concentration which scavenges 50% of DPPH after 1 h. The efficient concentration was obtained by interpolation on a calibration curve (Saha *et al*., 2013).

2.6 Chromatographic analyses

2.6.1 Transesterification of the fatty acids

The fatty acid composition was determined by gas chromatography with flame ionization detection (GC-FID). Into a dry Sovirel tube, 10 mg of oil (extracted from the seeds) was weighed and 200 µL of *n*-hexane and 500 µL of BF_3 reagent (methanol/14% BF_3/*n*-hexane (55:25:20) were added. The sealed tubes were placed in a water bath at 70°C for 1.5 h. After transesterification, 500 µL of a saturated solution of sodium chloride NaCl (40%) and 200 µL of sulfuric acid (10%) were added to the tube and the mixture was well shaken. Then 7 mL of *n*-hexane was added and the tube was shaken vigorously. Into the instrument, 1 µL of the supernatant was injected and the flame was analysed using a gas chromatograph Agilent HP 6890 Palo Alto, CA (USA) equipped with RT-2560 capillary column (100 m length, 0.25 mm i.d., 0.20 µm film thickness). Nitrogen was used as carrier gas at a flow rate of 1.6 mL min^{-1}. The initial oven temperature was held at 170°C for 2 min, increased at a rate of 3°C min^{-1} ramp to 240°C and finally held there for 15 min. The injector and detector temperatures were 225°C. Individual fatty acids were identified by comparing their retention times with a certified FAME mix. Quantification was based on relative peak area ratio.

2.6.2 Gas chromatography-mass spectrometry (GC-MS) analyses of fatty acid

GC–MS analysis was performed on a gas chromatograph HP 6890 (II) interfaced with a HP 5972 mass spectrometer with electron impact ionization (70 eV) in the same conditions as the GC-FID analysis. The carrier was helium gas at a flow rate of 1.2 mL min^{-1}. Each sample (1 µL) was injected in the split mode (1:20). The components were identified by comparing their relative retention times to the one of the FAME standards and their mass spectra with the data from the Wiley, Mass-Finder and Adams GC-MS libraries (Bettaieb *et al*., 2019).

2.6.3 HPLC-DAD of phenolic compounds

Analyses were performed on an HPLC system (Shimadzu Nexera XR) equipped with a pump, auto-sampler, column oven, and diode-array UV/VIS detector. The separation was executed on a Zorbax 300 SB-C18 column (4.6 µm, 150 mm, 3.5 µm). The mobile phase was composed of water + 0.1% HFO and CN + 0.1% HFO with the gradient elution system at a flow rate of 1.0 mL/min. The injected volume was 20 µL. The detection UV wavelength was set between 190 to 390 nm. The column temperature was set at 25°C.

Standard solutions of 25 phenolic compounds were prepared in methanol-water (80/20; v/v) at the concentration of 1 mg/mL, except gallic acid (1.2 mg/mL), chlorogenic acid (1.2 mg/mL), rutin (0.3 mg/mL) and rosmaric acid (1.7 mg/mL). Samples were shaken vigousrously and filtered through a 0.45-µm filter before injection. The results reported are an average and standard deviation calculated from at least three trials. All 25 phenolic compounds were used without further purification.

2.7 Indexes calculations

The Atherogenic Index (AI) and Thrombogenic Index (TI) were calculated according to the formulas described by Ulbricht and Southgate (1991). AI and TI indices are indicators of the potential effect of fats on the prevention of atherosclerosis, thrombosis and cardiovascular health.

$$AI = \frac{(C12:0 + 4*C14:0 + C16:0)}{[\sum MUFA + \sum PUFA\,(n-3)\,and\,(n-6)]} \quad (3)$$

$$TI = \frac{(C14:0 + C16:0 + C18:0)}{[0.5*\sum MUFA + 0.5*\sum PUFA\,(n-3) + 0.5*\sum PUFA(n-6) + (n-3)/(n-6)]} \quad (4)$$

2.8 Statistical analysis

Each sample was analysed at least in triplicate. The mean and standard deviations were calculated from these values using Microsoft Excel 2013. Two way analysis of variance (ANOVA) tests were carried out with replication. Tukey test was conducted to determine the significant difference among the samples based on a 95% confidence level ($p < 0.05$).

3. Results and discussion

3.1 Oil extraction yield

The time of extraction by ultrasound method was choosen according to the study done by Perrier *et al*., 2017. The yields of oil extracted from *A. polycantha* and Neem seed powders by differents methods are given in Table 1.

Table 1. Yield (in %) of oil extracted using different solvents by different methods

Seed	Method of extraction	Solvent			
		Hexane	Isopropanol	Acetone	Ethanol
Acacia polycantha	Soxhlet	7.3 ± 0.3^{aA}	9.3 ± 0.4^{bA}	7.66 ± 0.02^{aA}	12.5 ± 0.4^{cA}
	Ultrasound	6.3 ± 0.3^{aBD}	7.8 ± 0.2^{bdB}	6.4 ± 0.1^{aB}	8.6 ± 0.6^{cdBD}
	Microwave	6.2 ± 0.3^{aCD}	6.6 ± 0.2^{adC}	7.4 ± 0.3^{bdeA}	8.1 ± 0.5^{ceCD}
Neem	Soxhlet	52.3 ± 0.3^{aA}	51 ± 2^{abA}	52 ± 2^{aA}	59 ± 5^{acA}
	Ultrasound	45.4 ± 0.5^{aA}	48.1 ± 0.7^{beB}	48.5 ± 0.7^{ceBC}	21 ± 1^{dBD}
	Microwave	51 ± 9^{aA}	44.2 ± 0.8^{aC}	49.4 ± 0.3^{aAC}	16.8 ± 0.5^{bCD}

Note: lowercase are used for the comparison between different solvents and the same method of extraction (the same line), and the upper case are used for the comparison between the same solvent

and different methods of extraction (the same column). Values with the same lowercase or upper case letter are not significantly different at the 5% significance level according to the Tukey test.

The yields of Neem seed extracts varied between 59% and 21%. Ethanol was the most effective solvent for the extraction (59% extraction yield with Soxhlet). The smallest yield of extraction was also obtained with ethanol in the microwave extraction. It should be noted that the other solvents have comparable extraction yields. By comparing the extraction methods, it can be noted that the Soxhlet method gave the best results (yield > 51%) followed by the microwave-assisted method (yields in three solvents > 44%) and finally the ultrasound-assisted method. The result of microwave is comparable to the result obtained with the Soxhlet method. These results can be explained by the very long time of Soxhlet extraction (18 h) which leads to several cycles of oil extraction. Comparing solvents during Soxhlet extraction, it can be noted that the best solvent in the Soxhlet extraction was ethanol. Thus the ethanol can replace the *n*-hexane for the extraction of oil. These extraction yields (in %) are quite high compared to those generally reported in the literature concerning the extraction of Neem seeds: 30% yield for Neem seeds from the Ivory Coast (Gosse *et al*., 2005) and 32% for Neem seeds from Senegal (Faye, 2010) and even much lower (15.4-23.8) for the seeds from Mexico (Munoz-Valenzuela *et al*., 2007). This oil content can be explained by the environmental conditions of tree growth, tree genotype and geographical origin. From the statistical results, there was no significant difference (at p = 0.05) between the yields in solvents used in Soxhlet extraction. The same result was obtained in the case of microwave extraction. There was also no difference in the case of the three methods with *n*-hexane and acetone as solvents.

The highest yield of oil in case of *A. polycantha* was obtained with ethanol (12.5 ± 0.4) by the Soxhlet extraction method. The lowest yield of extraction was obtained with *n*-hexane (6.2 ± 0.3) by the microwave method. Ethanol was the most effective solvent for oil extraction with a maximum yield of 12.5% followed by isopropanol (9.3%), acetone (7.8%) and *n*-hexane (7.3%). Despite the relatively shorter time of extraction compared to the other methods, a high yield of extraction was obtained. These new methods of oil extraction can replace the classical extraction (Soxhlet) method with great savings in time and energy. It can also be noted that isopropanol or ethanol can replace *n*-hexane in the extraction of oils. The oil yield of *A. polycantha* is comparable to the yield obtained on *Acacia cyclops* (6.83 ± 1.26%), *Acacia ligulata* (9.04 ± 2.34%), *Acacia salicina* (12.18 ± 3.56%), *Acacia cyanophylla* (10.17%), *Acacia tortilis* (4%), and *Acacia mollissima* (7.16%) (Banerji *et al*., 1988; Khan *et al*., 2012; Youzbachi *et al*., 2012, 2015). This result is also comparable to those obtained by Perrier *et al*. (2017) which showed that isopropanol can replace hexane in oil extraction. This yield is low compared to the results observed by Perrier *et al*.,(2017) and by Zhong *et al*., (2018) but higher than those obtained by Zhan-jun *et al*. (2016) and by Bettaieb *et al*. (2019). At p = 0.05, there was no significant difference between the yields of acetone and *n*-hexane extracts using the Soxhlet and ultrasound-assisted methods. The same result was obtained in the case of microwave in three solvents (*n*-hexane, acetone and isopropanol). There was also no difference in the case of the three methods with *n*-hexane as solvent.

3.2 Total Phenolic Content (TPC)

The results of TPC evaluated by the Folin-Ciocalteu method are presented in Table 2.

Table 2. Total Phenolic content (TPC) of oils extracted in different solvents by different methods

Seed	Method of extraction	TPC (mg GAE/g of oil extracted in solvent)			
		Hexane	Isopropanol	Acetone	Ethanol
Acacia polycantha	Soxhlet	5.36 ± 0.08^{aA}	13.04 ± 0.03^{bA}	12.01 ± 0.08^{cA}	31.5 ± 0.2^{dA}
	Ultrasons	5.96 ± 0.06^{aB}	7.92 ± 0.03^{bB}	10.15 ± 0.03^{cB}	15.77 ± 0.02^{dB}
	Microwave	8.88 ± 0.01^{aC}	4.22 ± 0.01^{bC}	6.57 ± 0.02^{cC}	17.12 ± 0.02^{dC}
Neem	Soxhlet	19.3 ± 0.2^{aA}	24.07 ± 0.06^{bA}	21.64 ± 0.02^{cA}	27.02 ± 0.05^{dA}
	Ultrasound	16.80 ± 0.05^{aB}	24.01 ± 0.07^{bA}	27.53 ± 0.04^{cB}	37.5 ± 0.2^{dB}
	Microwave	9.36 ± 0.02^{aC}	18.50 ± 0.04^{bB}	17.43 ± 0.08^{cC}	30.30 ± 0.05^{dC}

Note: lowercase are used for the comparison between different solvents and the same method of extraction (the same line), and the upper case are used for the comparison between the same solvent and different methods of extraction (the same column). Values with the same lowercase or upper case letter are not significantly different at the 5% significance level according to the Tukey test.

For Neem oil, the TPC varied between 9.36 ± 0.30 and 30.30 ± 0.50 mg GAE/g of oil. The maximum TPC was obtained in ethanol followed by acetone, then isopropanol and *n*-hexane. This can be explained by the fact that phenolic compounds are more soluble in polar solvents (ethanol > isopropanol > acetone > *n*-hexane). Ethanol can therefore be used as solvent for extraction because it is less toxic to the environment and less expensive compared to other the solvents (Shewale and Rathod, 2018). Using ethanol as the solvent, the best method of extracting phenolic compounds was the ultrasound followed by the microwave and then the Soxhlet method. This result can be explained by the fact that ultrasound promotes a better penetration of the solvent into the cells tissues, thus causing shearing of tissues which leads to the liberation of oil. The results of TPC of oil for all the methods and solvents used were statistically different (at the p = 0.05 level of confidence).

For *A. polycantha*, the TPC varied between 5.36 ± 0.3 and 31.48 ± 0.4 mg GAE/g of oil. The lowest value was obtained with *n*-hexane and the highest value was obtained with ethanol. It appears that among the oils, that extracted in ethanol had the highest content of phenolic compounds than those extracted in *n*-hexane. This is as an apolar solvent that can extract only lipophilic compounds while ethanol or water are much more polar and can extract such polar compounds as phenolics. It can be noted that ethanol gives better results followed by acetone, isopropanol and finally *n*-hexane. These results agree with those of literature (Bettaieb *et al*., 2019; Fathi-Achachlouei *et al*., 2019). Compared to Virgin olive oils, *A. polycantha* oils are very rich in phenolic compounds. In the classification of Italian Virgin olive oils regarding tatste perception (TPC as "low" with 50-200, "medium" with 200-500 and "high" with 500-1000 mg GAE/kg), the total phenolic content is very high in *A. polycantha* and Neem oils (Kalogeropoulos and Tsimidou, 2014) . The results of TPC of extracts for all the methods and solvents used were significantly different (at p = 0.05).

3.3 Antioxidant activity

The antioxidant activity (CE_{50}) of the oils was also evaluated and is prensented in Table 3. This activity was compared to that of oils purchased on the market and which were extracted artisanally.

Table 3. Antioxidant activity of oils extracted in different solvents by different methods

Seed	Method of extraction	Antioxidant activity (CE_{50} in mg/mL) of oil extracted in solvent			
		Hexane	Isopropanol	Acetone	Ethanol
Acacia polycantha	Soxhlet	17.93 ± 0.02^{aA}	13.54 ± 0.01^{bA}	7.76 ± 0.01^{cA}	2.52 ± 0.04^{dA}
	Ultrasound	22.74 ± 0.03^{aB}	12.40 ± 0.06^{bB}	12.13 ± 0.03^{cB}	3.23 ± 0.02^{bB}
	Microwave	22.81 ± 0.02^{aC}	29.66 ± 0.04^{bC}	16.85 ± 0.05^{cC}	16.26 ± 0.01^{dC}
Neem	Soxhlet	15.82 ± 0.01^{aA}	10.95 ± 0.03^{bA}	11.50 ± 0.02^{cA}	12.48 ± 0.05^{dA}
	Ultrasound	22.74 ± 0.04^{aB}	12.34 ± 0.02^{bB}	11.78 ± 0.05^{cB}	2.71 ± 0.01^{dB}
	Microwave	22.91 ± 0.02^{aC}	12.92 ± 0.03^{bC}	14.91 ± 0.01^{cC}	1.95 ± 0.01^{dC}
oil extracted artisanally, purchased on the market		17.79 ± 0.08			
BHT		17.05±0.03			

Note: lowercase are used for the comparison between different solvents and the same method of extraction (the same line), and the upper case are used for the comparison between the same solvent

and different methods of extraction (the same column). Values with the same lowercase or upper case letter are not significantly different at the 5% significance level according to the Tukey test.

The antioxidant activity varied between solvents and from one method to another. This table shows that the CE_{50} varies from 1.95 to 22.91 mg/mL for Neem oil and from 2.52 to 29.66 for *A. polycantha* oil. The CE_{50} of the standard used (BHT) was 17.05 mg/mL. It should be noted that the lower the CE_{50}, the higher the antioxidant capacity of the oil. The solvent with the highest antioxidant activity was ethanol. Neem oil extracted from ethanol showed the highest activity and that from *n*-hexane the lowest activity. The oil from ethanol had a good CE_{50}, followed by that from acetone, then isopropanol and *n*-hexane. The results are consistent with those of the analyses of phenolic compounds obtained in different solvents.

The most efficient method to obtain a good antioxidant activity was the microwave. This result is better than those obtained with isopropanol, acetone and ethanol extracts but was comparable to the *n*-hexane extract. In the case of the oil obtained from *A. polycantha*, the best antioxidant activity was obtained for ethanol extract (3.23 mg/mL) using ultrasound as method of extraction. It can be noted that the extracts from all solvents exhibited good antioxidant activities than the standard used (BHT) except *n*-hexane. Even though *n*-hexane gave higher extraction yields (%), the oil obtained with this solvent did not have a good antioxidant activity in general (CE_{50} being higher than that of BHT). The oil with better antioxidant activity was obtained from the seeds of *A. polycantha.* Statistically, the CE_{50} of the oils extracted using all the methods and solvents were significantly different (at p = 0.05).

3.4 GC-MS and GC-FID of fatty acids methyl esters (FAME)

The GC-FID chromatogram of fatty acid of oil extracted from *A. polycantha* seeds using ethanol is shown in Fig. 1. The profiles of the oils with other solvents showed similar peak patterns; only the compounds and the quantities varied, as it can be seen in Table 4 for Neem oil and Table 5 for *A. polycantha* oil.

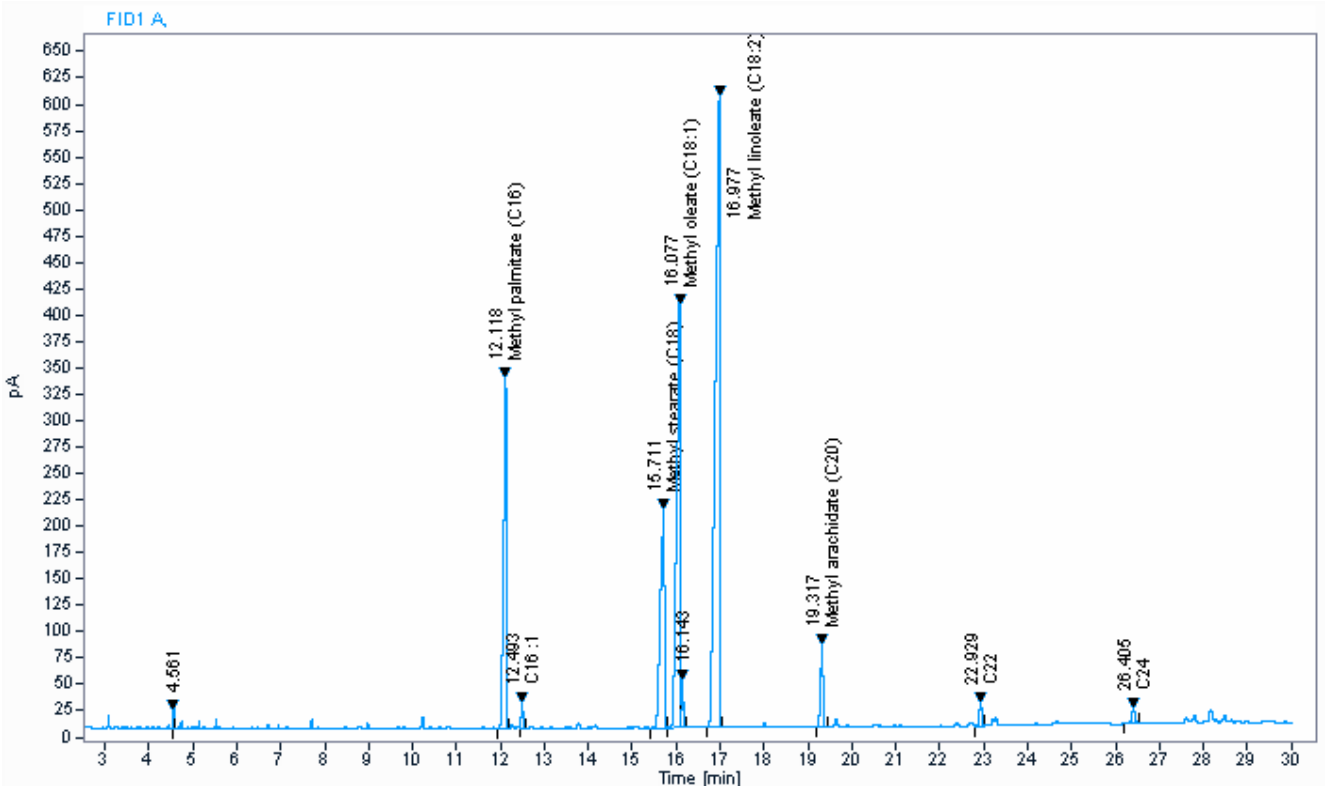

Figure 1. GC-FID of fatty acids of *Acacia. polycantha* oil extracted with ethanol and ultrasound method

Table 4. Fatty acid of Neem oil

Identified compound	Proportion (%)											
	Soxhlet				Ultrasound				Microwave			
	Hex	Iso	Ace	Eth	Hex	Iso	Ace	Eth	Hex	Iso	Ace	Eth
Palmitic acid (C16:0)	18.54	18.40	18.78	18.40	18.49	18.26	18.58	18,62	18.54	19.78	18.56	20.54
Margaric acid (C17:0)	/	/	/	/	/	0.17	/	0.18	0.17	/	0.18	/
Stearic acid (C18:0)	16.39	18.48	15.95	18.58	18.90	18.84	17.13	16.58	19.01	18.18	19.05	15.94
Oleic acid (C18:1)	37.94	40.95	42.64	40.67	41.28	40.67	41.16	40.52	41.02	40.77	40.75	41.79
Linoleic acid (C18:2)	16.89	18.98	20.56	19.36	19.27	19.73	20.57	22.02	19.01	18.57	19.00	19.70
α-Linolenic acid (C18:3)	0.54	0.75	0.98	0.67	0.57	0.63	0.75	0.86	0.58	0.75	0.61	0.82
Arachidic acid (C20:0)	1.17	1.39	1.10	1.48	1.48	1.53	1.27	1.23	1.53	1.35	1.52	0.96
cis-13,16-docosadienoic acid (C22:2)		FID										
Lignoceric acid (C24:0)	4.33	0.48	/	/	/	/	/	/	/		/	/
SFA	40.43	38,75	35.83	38.46	38.87	38.8	36.98	36.61	39.25	39.31	39.31	37.44
USFA	55.40	60.68	64.18	60.7	61.12	61.03	62.48	63.40	60.61	60.09	60.36	62.31
MUFA	37.97	40.95	42.64	40.67	41.28	40.67	41.16	40.52	41.02	40.77	40.75	41.79
PUFA	17.43	19.73	21.54	20.03	19.84	20.36	21.32	22.88	19.59	19.32	19.61	20.52
USFA/SFA	1.37	1.57	1.67	1.57	1.57	1.57	1.69	1.73	1.54	1.53	1.54	1.66
IA	0.33	0.30	0.29	0.30	0.30	0.30	0.30	0.29	0.31	0.33	0.31	0.33
IT	1.26	1.21	1.08	1.22	1.22	1.21	1.14	1.11	1.24	1.26	1.24	1.17

SFA: saturated fatty acids; USFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids, FID: detected only by GC-FID; Hex: hexane; Iso: isopropanol; Ace: acetone; Eth: Ethanol; /: not detected. IA: Index of Atherogenicity, IT: Index of Thrombogenicity.

Average values were obtained on three replicates; standard deviations were < 0.001 for all values.

Table 5. Fatty acids of *Acacia polycantha* oil

Identified compound	Proportion (%)											
	Soxhlet				Ultrasound				Microwave			
	Hex	Iso	Ace	Eth	Hex	Iso	Ace	Eth	Hex	Iso	Ace	Eth
Palmitic acid (C16:0)	13.82	14.52	13.88	14.59	14.47	14.63	14.61	16.15	14.94	13.91	15.80	15.54
Palmitoleic acid (C16:1)	0.82	0.78	0.79	0.84	0.85	0.82	0.82	0.83	0.87	0.67	0.90	/
Stearic acid (C18:0)	17.42	17.04	17.70	16.91	17.39	17.28	17.55	16.06	17.52	17.39	17.02	14.54
Oleic acid (C18:1)	25.02	26.32	24.98	25.26	25.43	26.65	25.31	26.07	25.51	26.74	25.79	26.12
Linoleic acid (C18:2)	36.41	36.29	35.94	35.40	36.34	35.53	35.88	37.07	35.66	35.66	36.53	32.97
α-Linolenic acid (C18:3)	/	/	/	0.18	/	/	/	/	/	FID	FID	/
Arachidic acid (C20:0)	4.81	4.00	4.83	4.41	4.35	4.09	4.63	3.26	4.35	4.39	3.26	2.81
Behenic acid (C22:0)	FID	FID	FID	1.21	1.16	1.01	1.20	0.56	1.16	1.24	FID	/
Lignoceric acid (C24:0)	FID	FID	FID	FID	FID	/	FID	FID	FID	FID	FID	/
SFA	36.05	35.56	36.41	37.12	37.37	37.01	37.48	36.03	37.97	36.93	36.08	32.89
USFA	62.25	63.39	61.71	61.68	62.62	63.00	62.01	63.97	62.04	63.07	63.22	59.09
MUFA	25.84	27.1	25.77	26.10	26.28	27.47	26.13	26.90	26.38	27.41	26.69	26.12
PUFA	36.41	36.29	35.94	35.58	36.34	35.53	35.88	37.07	35.66	35.66	36.53	32.97
USFA/SFA	1.73	1.78	1.69	1.66	1.68	1.70	1.65	1.78	1.63	1.71	1.75	1.80
IA	0.22	0.23	0.22	0.24	0.23	0.23	0.24	0.24	0.24	0.22	0.25	0.26
IT	1.00	1.00	1.02	1.02	1.02	1.01	1.04	1.01	1.05	0.99	1.04	1.02

SFA: saturated fatty acids; USFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids, FID: detected only by GC-FID; Hex: hexane; Iso: isopropanol; Ace: acetone; Eth: Ethanol; /: not detected. IA: Index of Atherogenicity, IT: Index of Thrombogenicity

Average values were obtained on three replicates; standard deviations were < 0.001 for all values.

Tables 4 and 5 present fatty acid compositions of the oils extracted by the different methods and in different solvents.The analyses showed that the oils contain nine different fatty acids identified by comparaison to standards. In both cases, unsaturated fatty acids represented a majority of compounds in the oils extracted.

In the case of Neem oil, it can be noted that for all the solvents and the extraction methods used, the proportions of compounds identified were the same. Margaric acid was present in the oil extracted by ultrasound and microwave but was absent in the oil extracted using the Soxhlet method. Oleic (37.94 to 42.64%) and linoleic (16.89 to 22.02%) acids were the most abundant unsaturated fatty acids present in the oils, while palmitic (18.40 to 20.54%) and stearic (15.94 to 19.01%) acids were the most abundant saturated fatty acids present. α-linolenic acid was the least abundant unsaturated fatty acid present in all the oils and margaric acid was the least abundant saturated fatty acid. This fatty acid composition is comparable to those obtained in literature (Diedhiou 2017; Djenontin *et al*., 2012; Faye 2010; Gossé *et al*., 2005; Kaushik, 2002; Kaushik et Vir, 2000; Momchilova *et al*., 2007). The Neem oil obtained in this study is richer in fatty acids than that one obtained by Nde *et al*. (2015) from Neem (*A. indica*) oil. The ratio of unsaturated to saturated fatty acids (USFA/SFA) (1.37 to 1.73) obtained in this study is higher than the ratio obtained by Diedhiou (2017) (0.6) on *A. indica*.Thus, the oil obtained in this study was more unsaturated than the others. However, some minor differences in fatty acids were observed (Diedhiou 2017; Faye 2010).

The most efficient solvent was ethanol and the most efficient method of extraction was the ultrasound. Unsaturated fatty acids (USFA) were the major components of the total fatty acids. The highest USFA content was obtained on oil using acetone and the lowest was obtained with *n*-hexane as solvent using the Soxhlet method. In all cases, for any extraction method and solvent used, the USFA content was above 60%. Cancer and coronary heart diseases can be prevented by USFAs because they have excellent nutritional and physiological properties (Yan *et al*., 2016). Regarding IA and IT, the seeds extracted with ultrasound showed the lowest values for these two indexes, and also presented the highest ratio of USFA/SFA; thus, the oil obtained by this method had the most beneficial profile of fatty acids.

In the case of *A. polycantha* oil, it can be noted that for any solvent and method used, the proportions of the compounds identified were the same. Oleic (25.02 to 26.74%) and linoleic (35.40 to 37.07%) acids were the most abundant unsaturated fatty acids present in the oils, while palmitic (13.92 to 15.80%) and stearic (14.54 to 17.55%) acids were the most abundant saturated fatty acids. Certain compounds not detected by GC-MS were detected by GC-FID: lignoceric, behenic and α-linolenic acids. Palmitoleic acid (0.67-0.90%) was the least abundant unsaturated fatty acid present in all oils extracted and arachidic acid (2.81-4.81%) was the least abundant saturated fatty acid. In table 4, the content of lignoceric acid (C24:0) obtained by Soxhlet method using *n*-hexane as solvent was 4.33%; however, this value for other treatments was far less or not detectable. This can be explained by the fact that lignoceric acid can be extracted in the course of a long time of extraction. *n*-Hexane is a solvent generally used to extract fatty acids; that is why fatty acids were less or not present in oils with other solvents. Soxhlet is classical method for the oil extraction (long time of extraction); the other methods gave reduced times of extraction. The most effecient solvent for the extraction SFAs was *n*-hexane (37.97) using the microwave method. Ethanol was most effective in extracting USFAs (63.97) using the ultrasound method. The most abundant fatty acids were extracted with ultrasound. The ratio of USFA/SFA varied between 1.63 to 1.80; thus the oil contained more unsaturated fatty acids than saturated fatty acids. In all cases, for whatever extraction method and solvent used, the USFAs were greater than 60%. These results may be compared to those obtained by other researchers on certain acacia species. However, some differences were observed in the fatty acid content; others obtained some fatty acids in trace amounts such as C12:0 and C14:0 (Banerji *et al*., 1988; Khan *et al*., 2012; Youzbachi *et al*., 2012, 2015) but, in this study, lignoceric acid (C24:0) was found. Regarding IA and

IT, the oil extracted in isopropanol and microwave as method showed the lowest values for these two indexes.

The difference in fatty acids between the two oils was that Neem oil did not contain palmitoleic and behenic acid while *A. polycantha* oil did not contain margaric acid. By comparing the oils extracted with those bought on the market such as soybean oil (SBO), sunflower oil (SFO) and olive oil, there were some differences as well as similarities. Polyunsaturated acids and monounsaturated fatty acids were the richest components of vegetable oils. SBO and SFO were rich in unsaturated fatty acids. Monounsaturated fatty acid (MUSFA) and polyunsaturated fatty acids (PUSFA) representing respectively 28.0 and 25.9% for MUSFA; 61.3 and 58.6% for PUSFA (Kozłowska and Gruczyńska, 2018). In the case of Neem oil, MUSFA varied from 37.97 to 42.64% and PUFA varied from 17.43 to 22.88%. In the case of *A. polycantha* oil, MUSFA varied from 25.77 to 27.41% and PUFA varied from 32.97 to 37.07%. It can be noted that all the oils were rich in unsaturated fatty acids. SBO and SFO were richer in polyunsaturated fatty acid. SBO and SFO are therefore more easily oxidizable than the oils obtained because they are rich in compounds having two double bonds in fatty acids structure (Szterk *et al*., 2010). Linoleic acid, an unsaturated fatty acid present in SFO and SBO in higher concentration makes it more susceptible to oxidation. Due to the low concentration of this fatty acid in Neem and *A. polycantha* oil, these oils are stable to oxidation. The oil obtained can be compared to the Extra Olive Oil which contains high oleic acid concentrations, ranging from 56% to 84%; polyunsaturated fatty acids: linoleic acid ranging from 3.5% to 21% and linolenic acid < 1.5% (Lanza and Ninfali, 2020). The same composition of fatty acids was obtained in olive oil (Tsimidou *et al*., 2003).

3.5 HPLC-DAD of phenolic compounds

Figure 2 shows the chromatogram obtained for Neem seeds extracted with ethanol in the microwave method. It can be noted that the chromatograms of the oils with other solvents have the same shape as in Fig. 2; only the compounds varied, as can be seen in Table 6 for Neem seed and in Table 7 for *A. polycantha* seed oils. Retention times of samples were compared with the retention times of 24 reference phenolic compounds. All the experiments were carried out under the same conditions and the proportions of phenols were determined.

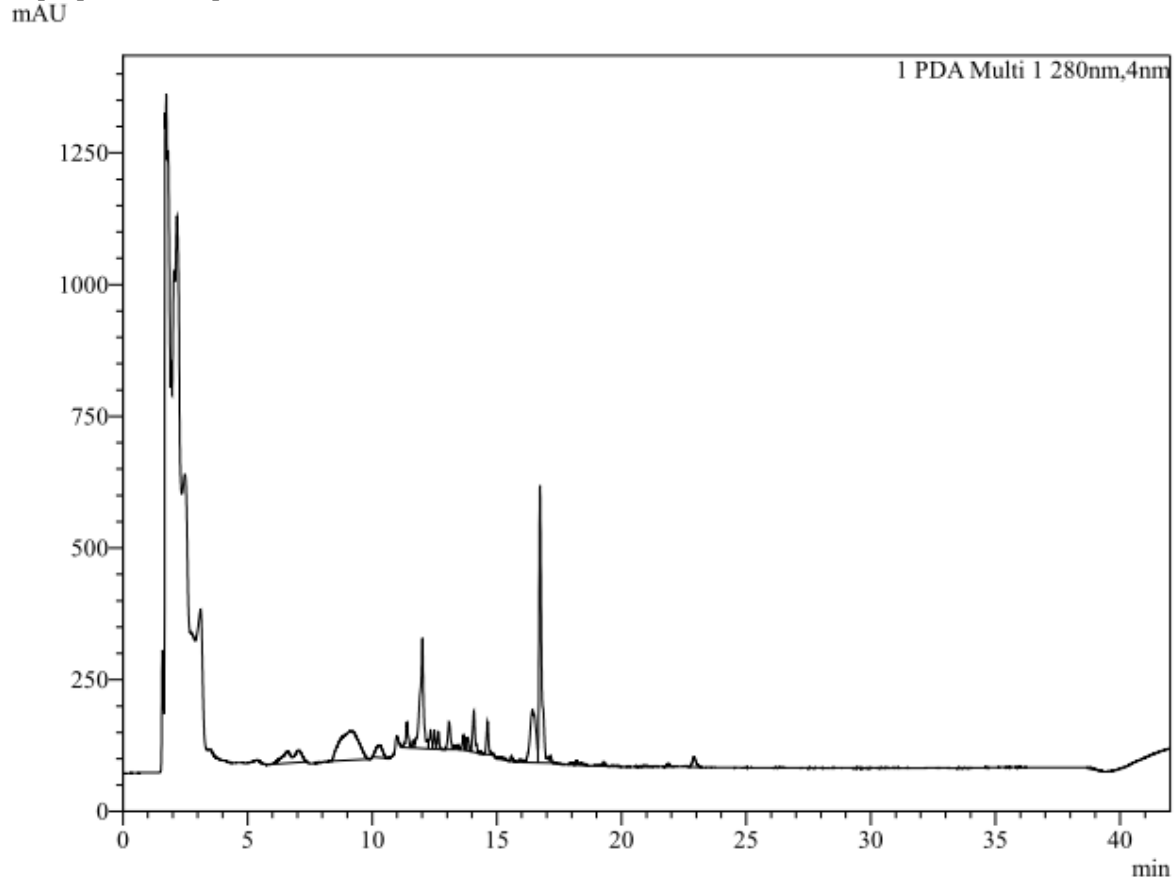

Figure 2. HPLC-DAD chromatogram showing phenolic peaks of *Acacia. polycantha* oil extracted with ethanol using the microwave method.

Table 6. HPLC-DAD analysis by comparison of retention time of phenolic compounds in Neem oil

Phenolic standard	Proportion (%) of Phenolic in oil											
	Soxhlet				Ultrasound				Microwave			
	Hex	Iso	Ace	Eth	Hex	Iso	Ace	Eth	Hex	Iso	Ace	Eth
1-Hydroxybenzoic acid	/	7.83	7.54	29.11	/	7.79	5.82	22.10	/	6.05	3.81	16.50
3-Hydroxybenzoic acid	37.81	50.45	62.01	/	37.43	/	52.28	/	30.66	/	46.76	/
Catechin	/	/	/	4.09	/	0.44	/	4.26	/	0.34	/	/
Vanillic acid	/	/	11.77	/	/	/	/	/	/	7.37	7.38	/
Chlorogenic acid	0.58	0.57	0.27	2.49	0.28	0.56	0.54	3.08	0.24	0.13	0.45	3.74
Syringic acid	/	/	/	/	/	/	0.06	/	/	/	/	0.83
Epicatechin	/	/	0.60	2.41	/	0.59	0.54	3.02	/	0.60	0.46	3.58
p-Coumaric acid	/	/	/	0.38	/	/	/	0.42	/	/	/	0.41
Transferulic acid	/	/	0.09	0.72	/	0.05	/	1.02	/	0.13	/	1.47
Ellagic acid	/	/	/	1.46	/	/	/	2.17	/	/	/	2.45
Rutin	/	/	3.48	/	/	/	/	/	/	/	/	/
Resveratrol	/	/	/	/	/	63.54	/	/	/	/	/	/
Cinamic acid	/	/	/	/	0.99	2.14	/	/	/	/	/	/
Flavan	/	19.44	25.37	47.72	/	19.38	20.31	60.84	/	19.44	13.44	30.75

/: not detected

Average values were obtained on three replicates; standard deviations were < 0.001 for all values.

Table 7. HPLC-DAD analysis by comparison of retention time of phenolic compounds in *Acacia polycantha* oil

Phenolic standard	Proportion (%) of Phenolic in oil					
	Soxhlet		Ultrasound		Microwave	
	Ace	Eth	Ace	Eth	Ace	Eth
Vanillic acid	16.22	/	9.36	94.96	/	13.62
Chlorogenic acid	0.49	1.57	0.22	/	/	0.26
Caffeic acid	0.47	/	0.15	/	/	0.29
Syringic acid	0.45	2.01	0.24	2.73	/	0.60
Epicatechin	0.58	/	/	/	/	/
p-Coumaric acid	/	/	/	/	/	0.06
Transferulic acid	/	/	/	3.88	/	1.86
Ellagic acid	47.11	/	/	/	1.72	/
Rutin	41.89	/	20.65	/	/	41.67

/: not detected

Average values were obtained on three replicates; standard deviations were < 0.001 for all values.

Figure 2 shows that the oils contained several phenolic compounds. However, *n*-hexane and isopropanol extracts of *A. polycantha* did not contain phenolic compounds for any of the extraction methods used. The *n*-hexane extract of Neem contained 3-hydroxybenzoic acid (37.81%) and chlorogenic acid (0.58%), even though many other compounds were not identified. Eight compounds were identified in the isopropanol extract of Neem oil by the ultrasound method, seven compounds by the microwave method and four compounds by the Soxhlet method. Resveratrol was identified only in oil extracted by ultrasound method using isopropanol. Syringic acid, resveratrol and cinamic acid were not identified in the extract by the Soxhlet method. These results can be explained by the fact that ultrasound promotes the better penetration of the solvent into the cell tissues, thus causing shearing which led to the liberation of molecules by the action of the solvent. For the acetone and ethanol extracts of Neem, almost the same composition was obtained for all the methods used. The compounds identifiend were 1-hydrobenzoic acid, catechin, vanillic acid, chlorogenic acid, epicatechin, *p*-coumaric acid, transferulic acid, ellagic acid, flavan and flavanone. Some of these phenolic compounds such as vanillic acid and *p*-coumaric acid had been identified by Gossé *et al*. (2005). The oil obtained can also be compared with olive oil. The polar fraction of olive oil contains more phenolic compounds than the Neem oil. In olive oil, the phenolic compounds identified were 4-acetoxy-ethyl-1, 2-dihydroxybenzene, 1-acetoxy-pinoresinol, apigenin, caffeic acid, cinnamic acid (not a phenol), *o*- and *p*-coumaric acids, ferulic acid, gallic acid, homovanillic acid, *p*-hydroxybenzoic acid, hydroxy-tyrosol, luteolin, oleuropein, pinoresinol, protocatechuic acid, sinapic acid, syringic acid, tyrosol, vanillic acid, and vanillin (Boskou *et al*., 2006b). The difference between these results can be due to the environmental conditions of tree growth, tree genotype and geographical origin.

The *A. polycantha* oil was not very rich in phenolic compounds, even though several peaks were not identified. The phenolic compounds identified were vanillic acid, chlorogenic acid, caffeic acid, syringic acid, epicatechin, *p*-coumaric acid, transferulic acid, sinapic acid, ellagic acid and rutin. Mambe *et al*. (2019), during the study of the leaf and bark extracts of *A.polyacantha*, had highlighted the presence of epicatechin and quercetin.

4. Conclusion

This study showed that Soxhlet extractions in ethanol afforded higher oil % yields. Ultrasound extractions gave the most suitable oils with higher total phenolic contents. The higher antioxidant properties were obtained with ultrasound. The most abundant fatty acids were oleic (an essential monounsaturated fatty acid), linoleic, stearic and palmitic acids in the case of Neem oil. The most abundant fatty acids were linoleic (an essential polyunsaturated fatty acid), oleic, stearic and palmitic acids. Regarding the solvents and methods used, the Neem oils obtained with acetone using ultrasound and Soxhlet, ethanol and microwave were the most interesting due to their high levels of MUFAs and PUFAs. In case of *A. polycantha*, higher proportions of MUFAs and PUFAs were obtained with ethanol as solvent by the microwave method. The phenolic compositions of oils extracted by ultrasound and microwave were not substantially different from those of oil obtained by the conventional solvent extraction method (Soxhlet). Ethanol can be used as green solvent for ultrasound-assisted extraction as a rapid method. From these results, it can be concluded that the oils obtained from the seeds of Neem and *A. polycantha* have a good fatty acid profile because of their high PUFA and MUFA, antioxidant, and phenolic contents. These oils thus have a high potential for use in the pharmaceutical, food and cosmetic industries.

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Conflict of interest: The authors declare that they have no conflict of interest.

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