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Fatty-acid Composition, Physicochemical Analysis and Biological Potential of Underutilized seed of *Leucaena leucocephala*

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Abstract

This study garnered vital information on the chemical and biological potential of *Leucaena leucocephala*, a seed considered underutilized in Nigeria. Fatty acid composition, physicochemical analysis of the oil, antioxidant and antimicrobial activities of the seed oil were carried out. Oleic acid, a monounsaturated fatty acid reported to aid in the prevention of cardiovascular and other health related disease is the most abundant fatty acids found in the seed oil, accounting for 51.18% of the total fatty acids. The seed oil shows appreciable low peroxide value of 2.24 ± 0.01 meqKg^{-1}, a clear indication of the presence of phytochemicals. The low free fatty acids value of the seed oil (8.46 ± 0.07 mgKOH/g) indicate the oil is of good quality. The high saponification value of 224.4 ± 0.41 mgKOH/g indicate suitability of the oil to be use in soap making, when blended with other oils, it can serve as a bioactive agent as the oil was found to be biologically active against diseases pathogens. The seed oil shows better potential at scavenging free radicals when compared to the reference standards ascorbic acid. The highest inhibition was recorded at a concentration of 50 µg/ml. *L. leucocephala* seed oil shows inhibition of 68.5±0.000% compared to 63.0±0.001 % for Ascorbic acid, as well as appreciable antimicrobial effect.

Keyword: Fatty acid composition, Physicochemical Analysis, Antioxidant, Antimicrobial, Fat and Oil

1. Introduction

The recent advances in natural product chemistry have enabled researchers to bridge the gap in the dearth of knowledge of seed oils potentials. Thus, a comprehensive analysis of the chemical composition, structural determination, the biological activities potential of natural products like vegetable oils can be performed (Okieimen *et al.*, 2005). *Leucaena leucocephala* commonly called wild tamarind is one of the seeds that are considered underutilized in Nigeria, owing to lack of prominent use among oil base industries and the populace. *L. leucocephala* is native to Central America and the Yucatan Peninsula of Mexico. It is now found naturalized in most tropical and subtropical areas of the world including Nigeria (Lim, 2012).

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According to Lim (2012), the fruit is a pod broadly linear (strap-shaped), thin, flat, 12–14 cm long by 1.5 cm wide, light green when immature, turns brown and dry when ripe and containing 15–30 seeds. She described the seeds as having an ovate-oblong or elliptic, flat, 6–10 mm long, shining, green when immature turning dark brown (Lim, 2012).

Studies conducted on the seeds in some part of the world showed that the seeds of *L. leucocephala* contain 6.4–7.5% oil, richly endowed with unsaturated fatty acids such as linoleic and oleic fatty acids (Nehdi *et al.*, 2014). Syamsudin (2010) reported *L. leucocephala* seeds have anti-inflammatory and antidiabetic activities.

This research aims to gather information on the fatty acid composition, physicochemical properties and biological potential of *Leucaena leucocephala*.

2. Materials and Methods

2.1 Source and Preparation of raw materials

Fully matured sample of *Leucaena leucocephala* was collected fresh from Kwara State, Nigeria. The plant material was identified at the Herbarium of Plant Biology, University of Ilorin, Ilorin, Nigeria where voucher specimen was deposited. The seeds were dried at room temperature, de-shelled and pulverized. This is kept in a cool dry place until needed for further laboratory analysis.

2.2 Extraction and Physicochemical Analysis of the oil

Oil extraction was carried out according to standard procedure described by Atolani *et al.* (2016) using n-hexane (1000 mL). 200 g samples each of grounded dried seeds was extracted using Soxhlet extractor at 55 ^{0}C for 7 hours. Physico-chemical parameters were carried out according to standard methods as described by Zubair *et al.*, (2018).

2.3 GC-MS Characterization of the Seed Oils and Modified Product

The esterified oil from the seeds was analysed using a Gas chromatograph (6890N, Agilent technologies network) coupled to an Agilent technology inert XL EI/CI Mass Selective Detector (MSD) (5975B, Agilent technologies Inc., Palo Alto, CA). Constituents were identified primarily based on the comparison of retention time with those of the authentic

standards and further confirmed by comparison of mass fragmentation pattern with those of NIST library (Atolani *et al.*, 2016).

2.4 Antimicrobial Activities of *Leucaena leucocephala* Seed Oils

Agar diffusion method was used to determine the antibacterial and antifungal properties of the oil (Atolani *et al.*, 2014). The oil was re-constituted in tween-20 at concentration range 62.5 to 250 mg/ml. Mueller Hinton agar were prepared as per the manufacturer's protocol. The sterile Mueller Hinton agar was poured into sterile Petri dishes and seeded with the six test bacteria (clinical isolates of *Salmonella typhi, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa*, *Klebsiella pneamananae* and *Staphylococcus aureus*) of Mcfarland standard. Sterile plates were impregnated with each oil solution. The impregnated discs was air-dried at room temperature and thereafter placed on the surface of the inoculated agar plates.

Four fungi (clinical isolates of *Candida albicans, Aspergillus nigar*, *Penicillium notatum* and *Rhizopus stolonifer*) were inoculated into mycological peptone and then incubated for one hour and these were then used to swab Sabouraud's Dextrose agar. The plates were incubated for 24 h (for bacteria strains) at 37 $^{\circ}$C and 48 h (for fungi strains) at 27 $^{\circ}$C. Erythromycin and Fluconazole will be used as the positive control for bacterial and fungi respectively while negative controls included disks impregnated with tween-20. The antimicrobial activity of the oils were evaluated at the end of the inoculated period by measuring the inhibition zone diameter in millimeters. The presence of zones of inhibition around each of well after the incubation period was regarded as the evidence of anti-microbial action while the absence of any measurable zone of inhibition will be interpreted as absence of antimicrobial activity.

2.4.1 Minimum Inhibitory Concentration (MIC)

The MIC values were evaluated according to published procedures of (Atolani et al., 2014). MIC was determined by dilution of the oil in medium and application on the dish using the disc diffusion method. Dilutions of the sample will be determined by incubating at 37 °C and fungi at 27 °C. The zone of inhibition was measured in mm after 24 or 48 h of growth, respectively. A control experiment was carried out by using an equal amount of sterile medium (only). The lowest concentration of the sample solutions that caused complete inhibition of the bacteria was taken as the MIC (Atolani *et al.*, 2014).

2.5 Free Radical Scavenging Activity by DPPH Assay

The ability of *Leucaena leucocephala* oil extracts from the seed to scavenge DPPH free radicals was assessed by the method of Ade-Omowaye *et al.*, (2015) and Birt *et al.*, (2002) with little

modification. The stock solution of the oil was prepared in dichloromethane to achieve the concentration of 100 µg/ml. Dilutions were made to obtain concentrations of 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390, 0.195 and 0.098 µg/ml. Diluted solutions, 2 ml each was mixed with 2 ml of dichloromethane solution of DPPH in concentration of 10 µg/ml. After 30 mins incubation in darkness at room temperature (25 ^{o}C), the absorbance was measured at 517 nm. Control solution contains equivalent concentrations of Ascorbic acid and all other reagents except the extract. Percentage inhibition was calculated using equation 1, whilst lC_{50} values were estimated from the % inhibition versus concentration plot, using a linear graph in Graph padprism 7. The data were presented as mean values ± standard deviation [n=3]

$$\%\ inhibition = \left(\frac{A of\ control - A\ of\ sample}{A\ of\ control}\right) \times 100 \quad (1)$$

3. Result and Discussion

3.1 Physicochemical Analysis of the Seed Oil

The Physicochemical characteristics of *Leucaena leucocephala* seed oil obtained via Soxhlet extraction are given in Table 1. The oil yield of the seed was 3.40 ± 0.02 %, similar to oil yield of 4.98% reported for *Prosopis africana by* Zubair *et al.* (2018). The oil shows an acid value of 16.83 ± 0.16 mgKOH/g which is more than the WHO/FAO value of 4 mgKOH/g recommended for edible oil. This value shows that the oil would be suitable for non-edible purposes. The appreciable low peroxide value of 2.24 ± 0.01 meqKg^{-1}, indicates the presence of phytochemicals. The low free fatty acids value of the seed oil (8.46 ± 0.07 mgKOH/g) indicate the oil is of good quality. The high saponification value of 224.4 ± 0.41 mgKOH/g the suitability of the oil to be use in soap making, when blended with other oils, it can serves as a bioactive agent as the oil was found to be biologically active against diseases pathogens as indicated in Tables 3 and 4. The low iodine value of the oil was further confirmed by GC-MS characterization which is given in Table 2. The iodine value of the oil is 101.52 ± 0.57 mgKOH/g which correlate to the low degree of unsaturation in the oil as indicated by a total degree of unsaturation of 51.18 %. This is in agreement with the work of Nehdi *et al.* (2014) who reported unsaturation level of 51.65% for the same oil.

Table 1: Physicochemical characteristics of *Leucaena leucocephala* seed oil

Parameters	*Leucaena leucocephala* seed oil
% Yield	3.40 ± 0.02
Saponification value (mgKOH/g)	224.4 ± 0.41
Acid value (mgKOH/g)	16.83 ± 0.16
Free fatty acid (mgKOH/g)	8.46 ± 0.07
Peroxide value (mgKOH/g)	2.24 ± 0.01
Ester value (mgKOH/g)	207.57 ± 1.12
Iodine value (mgKOH/g)	101.52 ± 0.57
Physical state at room temperature	Liquid

3.2 Fatty Acids Composition of Extracted Oils

The data for the fatty composition of the seed oil are presented in Table 2. Oleic acid, a monounsaturated fatty acid, widely reported to aid in the prevention of cardiovascular and other health related disease is the most abundant fatty acids found in the seed oil which account for 51.18% of the total fatty acids. Followed by palmitic acid (37.69 %), Stearic acid (8.99 %), Eicosanoic (1.66 %). Stearic acid and valeric acid make up (7.3%) and (1.96%) respectively in the oil. Tridecanoic acid (12:0) and Palmitoleic acid (16:1) were found in trace amount of 0.62 % and 0.87 % respectively.

Table 2: Fatty acids composition of *Leucaena leucocephala* seed oil

S/N	Fatty acids	Saturation	% composition
1	Tridecanoic acid	12:0	0.62
2	Palmitoleic acid	16:1	0.87
3	Palmitic acid	18:0	37.69
4	Oleic acid	18:1	50.31
5	Stearic acids	18:0	8.99
6	Eicosanoic acid	20:0	1.66
	Total Saturate		**48.96**
	Monounsaturate		**51.18**
	Polyunsaturate		
	Total Unsaturate		**51.18**

3.3 Antioxidant activities of the *L. leucocephala* seed oil and Ascorbic acid

Antioxidants are phytochemicals that could reduce the level of oxidative stress and reduce the oxidation of cells in the body (Ali *et al.*, 2008). Reaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH) provides an easy, rapid and convenient methods to evaluate the potential of seed oils to scavenge reactive chemical species in the body (Moţ *et al.*, 2011).

Result of the antioxidants potential of *Leucaena leucocephala* seed oil compared with the reference standards Ascorbic acid are presented in Table 3. Both the seed oil and Ascorbic acid shows a dose dependent inhibition against DPPH. The seed oil shows better potential at scavenging free radicals when compared to the reference standards ascorbic acid. The highest inhibition was recorded at a concentration of 50 µg/ml. *L. leucocephala* seed oil shows inhibition of 68.5±0.000 % compared to 63.0±0.001 % for Ascorbic acid. Both the seed oil and Ascorbic continues to show appreciative antioxidant effect as a fairly high antioxidant value of 64.3±0.000 % and 54.2±0.000 % at concentration dose of 0.391 50 µg/ml were recorded respectively.

This high antioxidant potential of *L. leucocephala* seed oil makes it a valuable addition to other less bioactive oils particular used in making antiseptic soap. It also shows the potential of the seed oil as a natural source of valuable antioxidants. The seed oil shows a lower IC_{50} value of 0.2463 µg/ml compared to the value of 0.2683 µg/ml for Ascorbic acid. This indicates the seed oil has a higher antioxidant potential than Ascorbic acid, as the lower the IC_{50} value, the higher the antioxidant potential (Karimi & Moradi, 2015). This study was found to be in line with study conducted by Chowtivannakul *et al.* (2016) in Malaysia who attributed the high antioxidant activities to *L. leucocephala* phenolic content

Table 3: DPPH Antioxidant activities of the *Leucaena leucocephala* seed oil and Ascorbic acid

Concentration (µg/mL)	% Inhibition of *Leucaena leucocephala* seed oil	% Inhibition of Ascorbic acid
50	68.5±0.000	63.0±0.001
25	67.9±0.001	62.6±0.001
12.5	67.2±0.000	61.7±0.000
6.25	67.0±0.000	60.7±0.001
3.125	66.6±0.001	60.4±0.000
1.563	65.3±0.000	58.8±0.000
0.781	64.6±0.000	57.8±0.000
0.391	64.3±0.000	54.2±0.000
IC_{50}	**0.2463**	**0.2683**

3.4. Antimicrobial activity of the *Leucaena leucocephala* seed oil

The diameter zone of inhibition of the test organisms by *Leucaena leucocephala* seed oil at different concentrations is shown in Table 4. The inhibition against similar organism by the control drugs (Erythromycin and Fluconazole), antibacterial and antifungal drugs respectively are presented in the Table 5.

The inhibition against all the test organisms ranges between 14.0 mm to 21 mm in diameter for the seed oil and 16 mm to 39 mm for the control drugs. The oil shows the highest zone of inhibition of 20 ± 0 mm compare to 34.5 ± 1.41 mm against bacterial by the control drug Erythromycin, similarly, highest zone of inhibition of 21.0 ± 1.41 mm compared to 39 ± 0 mm against fungi by the control drug Fluconazole. This was in agreement with the work of Aderibigbe *et al.* (2011) where the oil shows a similar dose dependent inhibitions.

The minimum inhibition concentration (MIC) value ranges from 3.125 to 6.25 mg/ml for the seed oil and 1.563 for the control drugs. The close MIC value is an indication that the seed oil is rich in phytochemicals with bioactive potentials.

Table 4: Inhibitory effect of *Leucaena leucocephala* seed oil against Pathogens

Pathogens	25mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL	1.563 mg/mL	MIC
S. aereus	20±0	17±0	14±0	12±0	-	3.125
B. subtilis	19±0	18±0	15±0	10±0	-	3.125
E. coli	20±0	17±1.41	14±1.41	-	-	6.25
P.aeruginosa	18±0	17±1.41	14±0	-	-	6.25
S. typhi	19±0	17±1.41	15±0	12±0	-	31.25
K.pnemonae	20±0	17±0	16±0	12±0	-	3.125
/R. stoloniter	21±1.41	17±1.41	14±1.41	10±0	-	3.125
P. notatam	20±0	17±0	14±1.41	10±0	-	3.125
C. albicans	20±0	18±1.41	15±1.41	13±1.41	-	3.125
A. niger	21±0	18±0	14±1.41	10±0	-	3.125

Table 5: Inhibitory effect of Control Drug (Erythromycin and Fluconazole)

Pathogens	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL	1.563 mg/mL	MIC
S. aereus	34±0	30±0	26±0	22±1.41	18±1.41	
B. subtilis	32±0	30±0	26±0	21±1.41	19±0	
E. coli	34±0	30±0	27±0	23±1.41	19±1.41	
P. aeruginosa	34±1.41	30±0	24±0	22±0	18±0	
S. typhi	34.5±0	32±1.41	29±0	26±1.41	16±0	
K. pnemonae	34±0	30±0	26±0	22±0	18±0	
R. stoloniter	38±0	36±0	32±0	30±0	27±0	
P. notatam	36±0	32±1.41	30±0	27±0	24±0	
C. albicans	39±0	38±0	36±0	32±0	28±0	
A. niger	38±141	34±0	32±0	30±0	22±0	

4. Conclusion

This research successfully garnered information on the fatty acids composition of *Leucaena leucocephala* seed oil. The potential to scavenge free radicals was compared to that of Ascorbic acid, a known antioxidant. Similarly, the ability of the seed oil to inhibit bacterial was compared to Erythromycin and fungi against Fluconazole, both of which are known antimicrobial agents

The oil shows appreciable low peroxide value of 2.24 ± 0.01 meqKg^{-1}, a clear indication of the presence of phytochemicals. The low free fatty acids value of the seed oil (8.46 ± 0.07 mgKOH/g) indicate the oil is of good quality. The high saponification value of 224.4 ± 0.41 mgKOH/g the suitability of the oil to be use in soap making, when blended with other oils, it can servce as a bioactive agent as the oil was found to be biologically active against diseases pathogens.

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