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PHYSICOCHEMICAL CHARACTERIZATION AND ANTIMICROBIAL PROPERTIES OF *NAUCLEA LATIFOLIA* (Smith) SEED OIL

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ABSTRACT

Nauclea latifolia (family: Rubiaceae) is a medicinal plant found in savannah region of tropical Africa and Asia. It is used traditionally in the management of various tropical diseases such as typhoid, malaria and hypertension. This study was carried out to characterize oil extracted from the seed of *Nauclea latifolia*. Physicochemical properties, antimicrobial activities and fatty acids composition of the oil sample were determined using standard procedures. The seeds were removed from ripe fruits of *N. latifolia*, ground into powder and extracted using Soxhlet apparatus at 67 °C for 2 h using n-hexane as the solvent. The oil yield was 11.10%. The oil was liquid at room temperature, had a fruity smell and pale yellow colour. pH and specific gravity of the oil were 5.02 and 0.975g/cm^3 respectively. Physicochemical parameters of the oil: acid value, iodine value, peroxide value and saponification value were 4.21mg/KOH/kg, 2.538mg/kg, 12.40 mEqO2/kg and 23.6425mg/KOH/kg respectively. The oil showed moderate zone of inhibition against *Streptococcus pyogene* (16mm) and *Staphylococcus aureus*(13mm) and a low zone of inhibition against *Pseudomonas aeruginosa* (9mm) and *Proteus spp* (11mm). The predominant fatty acids present in the oil were Palmitic acid (20.47%), Stearic acid (4.57%) oleic acids (23.46%), Linoleic acid (10.52%), Behenic acid (12.74%) and Erucic acid(1.60%). The ratio of saturated to unsaturated fatty acids was 0.8. The oil of *N. latifolia* showed moderate physicochemical and fatty acid properties. The study also revealed the potential of the oil in the treatment of infections caused by some of the tested microbes.

Keywords: *Antimicrobial, Characterization, Nauclea latifolia, Seed oil, Physiochemical*.

INTRODUCTION

Nauclea latifolia (family: Rubiaceae), [syn. *Sarcocephalus latifolius, Sarcocephalus russeggeri* Kotschy ex Schweinf, *Nauclea esculenta* (Afzel. ex Sabine) Merr], commonly known as cushion tree is a straggling shrub or small tree found in the savannah regions of tropical Africa and Asia (Gidado *et al*, 2005). It produces fleshy fruits which are embedded with numerous small brownish hard seeds surrounded by a pink, edible and sweet–sour pulp (Oyedeji-Amusamand Ashafa, 2019). *N. latifolia* is wildly distributed in Nigeria and found almost in every part of the country. It is called "Tafashiya or Tuwon-biri" in Hausa, "Opepe" in Yoruba and "Ubulumu" in Igbo.

Different parts of the plants are used traditionally for the treatment of various tropical diseases. The roots extracts of *Nauclea latifolia* are used for the treatment of hypertension, malaria, induce abortion and as a purgative (Antia and Okokon, 2014). Various scientific studies on the different parts of *N. latifolia* showed a wide range of biological activities such as antimicrobial, antiulcerative, anti-viral, anti-plasmodial, anti-trypanosomal, anti-diarrhoeal, antioxidant, anti-hypertensive properties (Balogun *et al*, 2018). Proximate analysis of the fruit showed it contains reasonable amount of crude protein, crude fibre, crude lipid and mineral elements (Onyekwere and Ernest, 2014).

Vegetable oils are important domestic commodities used in every household worldwide. These oils are used mainly for cooking, baking, frying and for industrial production of soap, margarine, paints and cosmetics (Olowokere *et al* 2019).

The most common sources of vegetable oils in Nigeria are peanut, palm oil and soybean. Other sources may include cottonseed, castor seed, sunflower, palm kernel etc. However, increase in population and high demand of vegetable oil as raw materials for some manufacturing industries, leads to the importation of cooking oil. As an effort to reduce this demand gab, researchers now focus on abandoned seed bearing plants in search of alternative sources of vegetable oils. *N. latifolia* fruits bear numerous seeds that may contain oil. However, to the best of our knowledge, there are limited or no information with regard to the oil extracted from the fruit of this plant. The present work is therefore aimed at extraction and determination of physicochemical properties, antimicrobial and fatty acids composition of oil from the seeds of *Nauclea latifolia.*

MATERIALS AND METHODS

Sample Collection and Preparation

Mature and ripe fruits of *Nauclea latifolia* were collected on Saturday 29th June, 2019 from a forest in Okehi L.G.A. Kogi State. The fruit sample was identified and authenticated by Mr. Gbenga Akande of the Herbarium unit, Department Biological Sciences, Federal University Lokoja, Kogi State Nigeria. A voucher number of 0128 was assigned. To remove the seeds, the fruits were allowed to rot and the seeds were washed in a bowl of water. The seeds were allowed to settle at the bottom of the bowl and the water was decanted. The seeds were then dried under shed for 24 hours. The dried seeds were pulverized into fine powder using laboratory motor and pestle. The powdered sample was kept in an air tight container until needed for analysis.

Extraction of Oil

Method of Zang *et al.,* (2017) was adopted for the extraction of oil. Powdered sample (200g) was packed into a porous thimble and extracted with 500mL of n-hexane using Soxhlet extractor for 2 hours. After extraction, the solvent was removed using rotary evaporator and the residual solvent was allowed to evaporate overnight.

Determination of Percentage oil Yield

The percentage oil yield was calculated using following relation (Aliyu *et al.,* 2017).

$$\% \text{ Oil yield} = \frac{\text{weight of oil}}{\text{weight of sample on dry matter bases}}$$

Physiochemical properties of the oil.

The oil extracted from of *N. latifolia* seed was analyzed for physicochemical properties. Standard procedures were employed to determine physical parameters such as iodine value, acid values, peroxide values and saponification value. Other physical characteristics such as color, ordour, pH and specific gravity were also determined as follow.

Determination of Acid Value

The oil sample (2 g) was transferred into a conical flask followed by addition of 50 mL absolute ethanol. The mixture was heated until the oil dissolved completely. After cooling, the oil was titrated against 0.1 M ethanol KOH using phenolphthalein as indicator. Appearance of a pink colour which lasted for about 30 seconds indicates the end point. Similar procedure was followed for blank titration (Wilberforce *et al.,* 2017).

The acid value was then calculated according to the following equation:

$$\text{Free Fatty Acid} = \frac{56.1 \text{ x } (a-b)M}{w}$$

Acid value = 2 x free fatty acid value

Where:

AV = Acid Value (mgKOH/g)

a = volume of KOH in mL for blank

b = volume of KOH in mL for test

M= molarity of KOH W= weight of the oil sample (g)

56.1 = molar mass of KOH

Determination of Saponification Value

The oil sample (0.5) was poured into a flask and 25 ml of KOH was added. The mixture was boiled for an hour using a reflux condenser, while the mixture is swirled regularly at different intervals until all the oil was completely saponified. Excess alkali was determined by titrating with 0.5 N HCl, 3 drops of phenolphthalein was used as indicator to a colourless end point. Blank was determined using KOH under same experimental procedure (Aliyu *et al*., 2017).

The saponification value of the oil was calculated as:

$$\text{Saponification Value} = \frac{56.1 \text{ x N X } (A - B)}{w}$$

N= Normality of KOH

A= Volume of HCL (ml) for the sample

B= Volume of HCL used (ml) for blank titration

W= Weight of sample taken (g)

56.1 = Equivalent weight = molecular weight of KOH

Determination of Iodine Value

The oil sample (0.5g) was weighed into a conical flask. 15mL of chloroform and 25mL of wiji's solution were added and the mixture was shaken properly. It was then covered tightly using a sheet of foil and kept in the dark for 30 minutes. To the resulting mixture, 20mL of 10% and 150mL of distilled water were added and titrated against 0.1 M $Na_2S_2O_3$ using starch as indicator. Disappearance of blue black colour of the mixture marks the end point (Zang *et al.,* 2017).

The Iodine value was calculated as follows:

$$\text{Iodine Value} = \frac{12.69 \text{ x N x } (V2 - V1)}{\text{weight of sample}}$$

Where

V_2 = volume of 0.1 N $Na_2S_2O_3$ needed by blank

V_1 = volume of 0.1 N $Na_2S_2O_3$ needed by sample

N = Normality of $Na_2S_2O_3$

Determination of Peroxide Value

The oil sample (0.5g) was placed in a flask followed by addition of 7.5 ml of acetic acid and 5 ml chloroform. The mixture was swirled properly until the sample was fully dissolved. 0.25 ml of saturated potassium iodide was added with vigorous shaking. Distilled (15 mL) water was added and the mixture was titrated with 0.05 N $Na_2S_2O_3$ solution until a pale yellow color appeared. Five mL of starch was then added and shaken until a blue color which indicates the endpoint was observed (Aliyu *et al.,* 2017). Peroxide value was calculated as:

$$\text{Peroxide Value} = \frac{2.6 \text{ x strenght of sodiumthiosulphate}}{\text{weight of sample}} \text{ x } 100$$

Determination of pH

The sample (2g) was placed in a dry clean beaker containing 13 ml distilled water (hot), it was stirred gently and allowed to cool at 25°C. pH meter was inserted into the mixture to obtain the pH of the oil (Garba *et al*., 2015).

Determination of Specific Gravity

A cleaned dry empty beaker was weighed and exactly 50 cm^3 of the oil sample was measured and poured into the beaker and reweighed. The weights of the 50cm^3 of the samples were recorded (Garba *et al.,* 2015).

The Specific gravity of the oil was calculated as:

$$\text{Specific gravity of oil sample} = \frac{\text{weight of oil sample}}{\text{volume of the oil sample}}$$

GC-MS Analysis of the oil sample

Preparation of Fatty Acid Methyl Esters (FAMEs).

The oil was heated in the presence of NaOH followed by addition of BF_3 methanol for esterification. To separate organic and aqueous layers, 5 mL of n-Heptane was added to the reaction mixtures with subsequent addition of NaCl solution. The organic phase, which is the n-heptane layer containing the esters was pipetted out and stored (-4^{o}C) for determination of fatty acid composition of the oil (Aliyu *et al.,* 2017).

GC-MS Analysis of the FAME

The analysis of the FAME was done using Shimadzu QP2010 Quadrupole (GC-MS) for the determination of fatty acid composition. The GC program includes raising oven temperature to 70^{o}C from ambient temperature using 10 degrees per minutes ramping rate. The initial and final column temperature was held for 0 and 5 minutes, respectively. The injector temperature was carefully programmed to 250°C. The identification of the unknown FAMEs were based on the similarity search performed using NIST 2005 Library on the basis of peak areas for Chromatography GC-MS was done using the SHIMADZU GCMS-QP 2010 data-handling program (Garba *et al.,* 2014).

ANTIMICROBIAL SCREENING

Microbiological Strains

Pure culture of the isolates used in this study were obtained from Ahmadu Bello University Teaching Hospital, Shika, Kaduna State Nigeria and were preserved in McCartney bottles with slant preparation of nutrients agar to maintain their growth.

Zone of inhibition

Fatty acid methyl ester was used for the study of antimicrobial properties of the oil. Microbial activity of the oil was tested using the disc diffusion method described by Collins *et al.* (2004) and fractions were prepared and tested using nutrient agar. Sterile 6 mm disc Whatmann number 1 filter paper discs were impregnated with varying concentrations of the fatty acid methyl ester: 100%v/v, 80%v/v, 60%v/v and 40%v/v. The bacterial cultures were inoculated on Nutrient Broth (Oxoid) and incubated for 24 h at 37°C. Adequate amounts of nutrient Agar (Oxoid) were dispensed into sterile plates and allowed to solidify under aseptic conditions. The bacterial cultures were adjusted to match McFarland turbidity standard. The test microorganisms were inoculated with a sterile swab on the surface of appropriate solid medium in plates. The agar plates inoculated with the test microorganisms were incubated for 1 hour before placing the fatty acid methyl ester impregnated paper disc on the plates. The bacterial plates were incubated at 37°C for 24 h, all plates were observed for zones of growth inhibition and the diameter of these zones was measured in millimeters. All tests were performed under sterile conditions.

Minimum Inhibitory Concentration (MIC)

Determination of the Minimum Inhibitory Concentration (MIC) was carried out according to the method described by Collins *et al.* (2004). A dilution series of the extract, ranging from 80%v/v, 60%v/v, 40%v/v, 20%v/v and 10%v/v were prepared and then transferred to the broth in micro tube. Before inoculation of the test organisms, the bacterial and yeast strains were adjusted to 0.5 McFarland and diluted nutrient Broth (Oxoid). The micro tubes were incubated at 37°C for 18 - 24 h for bacteria cultures. The MIC values of the extracts were defined as the lowest concentration that showed no growth (turbidity).

Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration (MBC) was determined by plating samples from clear test tubes showing no growth onto nutrient Agar. MBC was defined as the lowest concentration yielding negative subculture. Ciprofloxacin was used as the standard antibacterial agent to serve as positive control while DMSO was used as negative control (Khan *et al.,* 2001).

RESULTS AND DISCUSSION

Table 1: Physicochemical Properties of *Nauclea latifola* Seed Oil

Parameter	***N. latifola* Seed Oil**
Oil yield (%)	11.10
Ph	5.02
Specific Gravity (g/cm^3)	0.975
Colour	Golden yellow
Odour	Fruity sweet
Acid Value (mg/KOH/kg)	4.21
Iodine Value (mg/kg)	2.538
Peroxide Value (mEqO_2/kg)	12.40
Saponification Value (mg/KOH/kg)	23.6425

Physicochemical properties such as percent yield, colour, ordour, pH, Density, Peroxide value, Iodine value, saponification value and peroxide value of *Nauclea latifolia* seed oils were presented in Table 1.

The oil yield of *Nauclea latifolia* seeds obtained in this work was 11.1%. The oil was pale yellow with a characteristic fruity smell. The yield is higher than the value reported in *Persea Americana* (8.10%) (Adaramola *et al*., 2016). The value is comparable with 13.23% from *Nymphaea lotus* seed oil *(*Aliyu *et al.*, 2017) and lower than values (50%) reported in *Sesamum Indicum L*. (Mohammed & Hamza, 2010). Oil yield is an important variable used to determine whether or not the seed can be considered as source of oil for industrial applications. According to Akinoso and Raji (2011), seeds with oil yield greater than 17% are considered as oil seeds. The specific gravity and pH of the oil were found to be 0.976 g/cm^3 5.02 respectively.

The peroxide values of the oil obtained in this study was 12.40 mEqO_2/kg. The value is low compared to 16.08 obtained from palm oil (Birnin-Yauri and Garba, 2011) and lower than 5.37±0.13 reported for Carica *papaya* seed oils (Malacrida *et al.*, 2011). Peroxide value is a measure of the content of hydro-peroxides presence in oil and indication of susceptibility to rancidity of the oil due to auto-oxidation (Adaramola *et al.*, 2016). The maximum acceptable value of peroxide values set by the Codex Alimentarius Commission for groundnut seed oils is 10 meq KOH/g (Mohammed and Hamza, 2010).

The iodine values of the N. *latifolia* seed oils was 2.538mg/kg. this value is very low compared to 29.00 ± 0.16 g/100g reported for African star apple (Omeje *et al.*, 2019). Iodine value is the measure of the degree of unsaturation presence in the oil. Higher iodine value means high degree of unsaturation. The very low iodine value obtained in this work indicates that *N. latifolia* seed oils are highly saturated. this implies the suitability of using the oil as plasticizers and lubricants (Adaramola *et al.*, 2016).

Acid Value of the oil was found to be 4.21mg/KOH/kg. This value was low when compared to the value (17.41 ± 0.43 mg/KOH/kg) of oil extracted from of African star apple (Omeje *et al.*, 2019) and close to value (4.51±0.08) obtained from *Persea Americana* oil (Adaramola *et al.*, 2016). Acid value determines the susceptibility of oil to rancidity; the lower the acid value of oil, the few fatty acid it contains which makes it less exposed to rancidity (Wilberforce *et al.*, 2017).

The *N. latifolia* seed oils had a saponification values of 23.6425 which is low compared to values 138.98±1.77mgKOH/g and 186.81±161mgKOH/g reported for *Nymphaea lotus Nymphaea pubescens* seed oils (Aliyu *et al.*, 2017). Saponification value is an indication of application or usefulness of oil. Oil with high saponification value is more suitable for application such as the manufacture of soaps while high saponification in refined oil is an indication of contamination (Akinyede *et al.*, 2016). The relatively low saponification value of oil from *N. latifolia* seeds implies its poor suitability for the production of soaps and detergents (Adaramola *et al*., 2016).

Table 2: Antibacterial activity of *Nauclea latifolia* Seed Oil.

Bacterial Strain	Zone of inhibition (mm) at different concentrations (%v/v)					
	40	**60**	**80**	**100**	**+Ve Control**	**-Ve Control**
Streptococcus pyogenes	-	-	10	16	26	-
Staphylococcus aureus	-	-	8	13	24	-
Pseudomonas aeruginosa	-	-	-	9	20	-
Proteus spp	-	-	-	11	28	-

Key: +Ve = Positive, -Ve = Negative

Antimicrobial activity of the fatty acid methyl esters of *N. latifolia* seed oil was evaluated using agar disc diffusion method. The test microorganisms used in this study were; *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus spp*. The antimicrobial activity of the oil is concentration dependent. The oil shows considerable moderate zone of inhibition against *Streptococcus pyogenes* (16mm) and Staphylococcus *aureus* (13mm) (Gram positive bacteria) when compared with the standard antibiotic (ciprofloxacin) which serves as positive control and DMSO as negative control. A low zone of inhibition was observed for *Pseudomonas aeruginosa* (9mm) and *Proteus spp*. (11mm) respectively. This implies that *N. latifola* seed oil can be used for the treatment of infection caused by *Streptococcus pyogenes* and *Staphylococcus aureus* (Gram positive bacteria) if properly standardized. The low zone of inhibition indicates that *N. latifola* seed oil may not be effective for the treatment of infection caused by *Pseudomonas aeruginosa* (Gram negative bacteria).

The observed antimicrobial activity of the FAME of *N. latifola* seed oil may be attributed to the fatty acids composition of the oil. Antimicrobial activity against *Clostridium perfringens* and *Staphylococcus pyogens* was reported for fatty acids such as myristic, palmitic, stearic, oleic, and linolenic (Chandrasekaran *et al.*, 2008).

Table 3: Minimum Inhibitory Concentration (MIC) of Nauclea *latifolia* Seed oil

Bacterial Strain	Concentration of Oil (v/v %)				
	10	**20**	**40**	**60**	**80**
Streptococcus pyogene	+++	+++	++	O*	-
Staphylococcus aureus	+++	+++	++	+	O*
Pseudomonas aeruginosa	+++	+++	++	+	O*
Proteus spp.	+++	+++	+++	+	O*

(MIC); Key: O* = MIC, - = No Growth (No Turbidity), + = Low Growth, ++ = Moderate Growth, +++ = High Growth.

Minimum inhibitory concentration of the Fatty methyl ester of the oil sample is presented in table 3. The MIC of the oil observed to inhibit the growth of the all organism tested (*Streptococcus pyogene*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus spp*) at concentration of 80%v/v.

Table 4: Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of Nauclea *latifolia* Seed Oil

Bacterial Strain	Oil Concentration (%v/v)				
	10	**20**	**40**	**60**	**80**
Streptococcus pyogenes	+++	+++	++	+	**O***
Staphylococcus aureus	+++	+++	++	+	**O***
Pseudomonas aeruginosa	+++	+++	+++	+	+
Proteus spp	+++	+++	+++	+	**O***

(MBC/MFC); Key: O* = MBC/MFC, - = No Growth (No Turbidity), + = Low Growth, ++ = Moderate Growth, +++ = High Growth

The minimum Bactericidal/Fungicidal Concentration of fatty acid methyl ester of the oil is presented in table 4. MBC/MFC was carried out so as to know if the organism is completely exterminated or just inhibited by FAME. The MBC/MFC value was observed to exterminate the growth of *Staphylococcus aureus*, *Streptococcus pyogenes* and *Proteus spp* at concentration of 80%v/v and *Pseudomonas aeruginosa* shows resistance on the FAME.

Table 5: Fatty Acids Composition of *N. latifolia* Seed Oil.

Common Name	IUPAC Name	Saturation	Retention Time (min)	Composition (%)
Palmitic acid	Hexadecanoic acid	16:0	15.787	20.47
Stearic acid	Octadecanoic acid	18:0	16.576	4.57
Linoleic acid	9,12-Octadecadienoic acid	18:2	17.618	10.52
Behenic	Docosanoic acid	22:0	17.729	12.74
Oleic acid	9-Octadecenoic acid	18:1	18.266	23.46
Erucic acid	13-Docosenoic acid	22:1	19.339	1.60
Arachidic acid	Eicosanoic acid	20:0	18.393	5.80
SFA				55.10
UFA				44.90
MUFA				31.66
PUFA				13.24
UFA/SFA				0.81

Key: SFA = Saturated fatty acids, UFA = Unsaturated fatty acids, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids

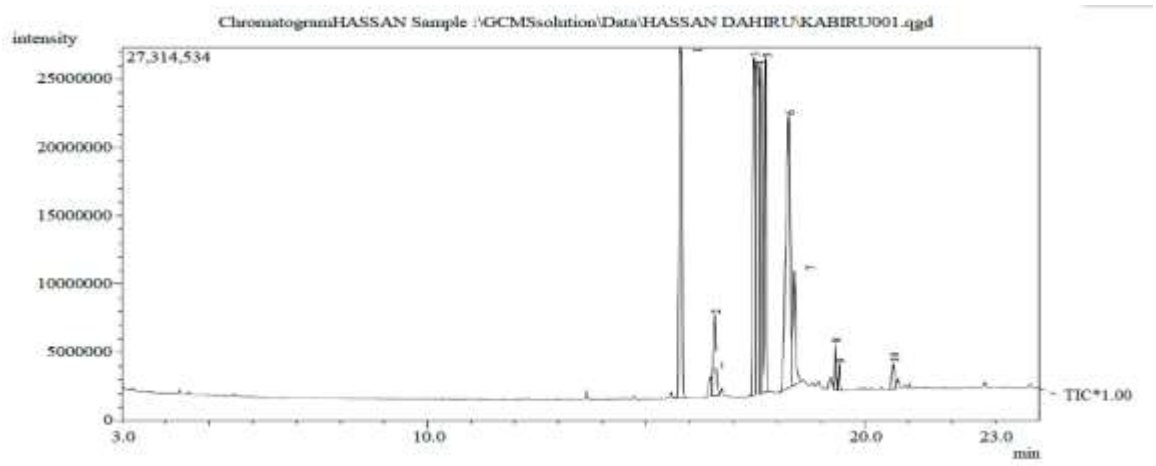

Fig 1: Chromatogram of *N. latifolia* seed oil.

The result of GC-MS analysis of *Nauclea latifolia* seed oil is presented in table 5. The predominant fatty acids in the extracted oil were oleic (23.46%), palmitic (20.47%), Behenic acid (12.74%) and linoleic (10.52%). Fatty acids present in the oil in low quantities include arachidic acid (5.80%), Stearic acid (4.57%) and Erucic acid (1.60%). The compositions of saturated fatty acids in the oil were 55.1% while that of unsaturated fatty acids were 44.9%. Monounsaturated fatty acids and polyunsaturated fatty acids were found to be 31.66% and13.24% respectively. This shows that the oil contains a higher percentage of saturated fatty acids than the unsaturated fatty acids.

Presence of some essential fatty acids such as linoleic acids and oleic acids implies the potential of the oil in preventing cardiovascular disorders such as coronary heart diseases and atherosclerosis as well as to prevents high blood pressure (Ajayi *et al.*, 2006).

CONCLUSION

Results obtained from the current study showed that oil yield of *Nauclea latifolia* seed was low (11.1%). The oil also showed moderate physicochemical properties which are useful for industrial and domestic application. The oil contains reasonable amounts of both saturated and unsaturated fatty acids. It also showed a promising antimicrobial activity particularly against *Streptococcus pyogenes* and this indicates that the oil may be useful for pharmaceutical formulation especially for the treatment of diseases caused by *Streptococcus pyogenes*. Further research is recommended to evaluate the domestic, pharmaceutical and industrial applications of the oil.

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