PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (1) 61-68 January 2024

Extraction Characterization of Oils Extracted from different Parts of Red and Yellow Varieties of *Anacardium occidentale* (Lin)

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ABSTRACT: The objective of this study was to extract and characterized the oils from different parts of red and yellow varieties of Anacardium occidentale after extractions using Soxhlet extractor and steam distillation techniques and characterization of the bioactive components using GC/MS analysis. Physicochemical parameters of the extracted oils were analyzed, and the bioactive compositions were also examined. The results showed that the oils extracted were within 0 and 0.5% moisture content and pH values ranged from 3.7 to 4.7. The average relative densities of the extracted oils by Soxhlet extraction and steam distillation at temperature 25^{o}C were 0.887 and 0.8745 g/cm^{3} respectively, with average saponification value of 132.45 mgKOH/g for Soxhlet extraction and 127.98 mgKOH/g for steam distillation. The iodine values of all the oils were between 35 and 19.00 mg iodine/100g; while the refractive index was between 1.7 and 1.9. The acid values were within the range of 10.00 and 13.00 mgKOH/g, and peroxide value of 1.67 and 1.20 mmol/l. The oils had low viscosity which were within 28 and 32 mpa.s. The compounds identified from the GC-MS results showed that cashew nut shell oil contained cardol, anacardic acid, cardanol, 2-methyl cardol, triacconten and β-sitosterol. The main bioactive components of the extracted oil identified from the stem bark, root bark, and leaf were alkaloids, tannins, flavonoids, coumarins, terpenoids, and saponins. This study, therefore, showed that Soxhlet extraction could be a better extraction method for extraction of oil from cashew nut shell which had higher relative density; while cashew stem bark, root bark and the leaf which had lower density were selective based on the target products.

DOI: https://dx.doi.org/10.4314/jasem.v28i1.7

Cite this paper as: NWOSU, N. B; OKORONKWO, N. E; NJOKU, D. K; EMOLE, P. O. (2024). Extraction Characterization of Oils Extracted from different Parts of Red and Yellow Varieties of *Anacardium occidentale* (Lin). *J. Appl. Sci. Environ. Manage.* 28 (1) 61-68

Dates: Received: 02 December 2023; Revised: 11 January 2024; Accepted: 21 January 2024 Published: 30 January 2024

Keywords: *Anacarduim occidentale;* Cashew Oil; Soxhlet Extraction; Steam Distillation

Anacardium occidentale is a fast growing, hardy and drought resistant multipurpose tree species cultivated in many tropical countries including Nigeria, It is one of the most well-known species for its nut, although all parts of the tree are useful. Healthy tree has oval leave 10 to 20cm long and up to 10cm wide reddish or light green while young and dark green when mature, the bark is grey (Orwa *et al.*, 2009). Many beneficial biological activities of cashew plants such as anticancer, antimicrobial, antioxidant, anti-diarrheal, analgesic, wound healing and insecticidal properties have been reported. Chemical composition and physicochemical properties of the leave, steam, root and true fruit have been reported to be of valuable commercial uses for food, medicine, industry and environment (Aremu and Akinwumi, 2014). The premier steps in isolating biologically active compound from plant resources include extraction, isolation, and characterization, toxicological and clinical evaluation. Thus, the study of *Anacardium occidentale* starts with extraction procedures that play a critical role in the extraction outcomes (Okoronkwo and Ifionu, 2016). Characterization and selection of extraction methods are dependent on the study objectives, sample and target compounds. Volatile substances are generally present at a low concentration

and must be extracted from the plant matrix before they can be analyzed. They are known to be thermally sensitive and vulnerable to chemical changes, thus adequate care is required in selecting an extraction method. Loss of some volatile compound, low extraction efficiency, degradation of unsaturated or ester compounds through thermal or hydrolytic effects and toxic solvent residue in the extract are some of the challenges encountered when using different extraction methods. To reduce these challenges, Soxhlet extraction and steam distillation methods are still widely accepted options in the laboratory (Okoronkwo, 2018*;* Danlami *et a*l., 2014). The prospects for cashew tree plantation are very high due to domestic and international demand for cashew tree products. The cashew shell oil represents about a quarter of the mass of an unshelled nut and approximate equal to that of the kennel (Rudiger, 1996). Cashew nut shell liquid (CNSL) is a cheap source of phenol (Ekpendu *et al.,* 2014). Cashew wood is used for termite resistant; some uses of CNSL include to prepare germicides, fungicide, insecticides and medicine including cancer treatment (Akande *et al.,* 2015). Cashew leaves are used for toothaches, gum problem and malaria. The stem of cashew consists of the bark and wood, the tea from the bark is used as a vaginal douche in Colombia, pulverized bark is soaked in water for twenty-four hours and used to treat diabetes. In Brazil and Nigeria, the bark is used to make an astringent decoction to treat toothache and inflammations of the gum. The back is also used in Ayurvedic medicine to detoxify snake bite, as well as for fevers, a laxative, to rid intestinal parasite (Leite *et al.,* 2016). The basic components of cashew tree that has the antiseptic, antibacterial, antifungal, anti-parasite allelopathic and medical properties are the phenolic compounds such as anacardic acid, cardanol, cardol and 2-methylcardol (Verma *et al.,* 2009). The rich presence of bioactive and other nutritive values supports the use of the different parts of *Anacardium occidentale* in ethno-medicine and equally creates the possibility for their use in drug formulation. (Nwosu *et al.,* 2023). Thus, this study characterized the oils extracted from different parts of varieties red and yellow of *Anacardium occidentale*.

MATERIALS AND METHODS

Study Area: The Samples used for the study were collected from Abia State University Uturu, Abia State Nigeria. Cashew samples from the selected sites were collected at different points per site. The first site was where the cashew yellow apples (CYA) are planted and the second site was the cashew Red Apple (CRA) plantation. At the first site (CYA), the bark, true fruit, leaves and roots were collected at random from different points within the plantation sites. The second site (CRA) the bark, true fruit, root and leaves were also collected in the same manner.

Sample preparation: The samples were tagged and bagged on site in clean colourless polyethylene bags to avoid contamination, and were then taken to the laboratory. The samples were air dried, ground, sieved through 2mm stainless steel sieve and were kept in an air tight container for further laboratory analysis.

Sample Extraction: The Soxhlet Extraction Method was used to extract oil from the different parts of the plant and Steam Distillation was done by the method described by Nichols (2019). Standard methods were used for the determination of physicochemical parameters of oils**,** relative density of an oil at t/20 ^{c}C is the ratio of the mass in air of a given volume 20 ^{o}C to that of the same volume of water at 20 ^{o}C was done by method described by Douglas *et al*., (2005). The saponification value of an oil which is the number of mg of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1 g of the sample was done by method described by Aslam, (2023). The iodine value which is a measure of the degree of unsaturation of the fatty acid in the oil and Refractive Index is ratio of velocity of light in vacuum to the velocity of light in the oil was done by method of Merv, (2011). The acid value is the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat and was done by method of Aslam, (2023). Peroxide value, a measure of the peroxides contained in the oil and viscosity is a measure of flow ability of oil at definite temperature as prescribed by Kim and Siang (2023).

GC-MS Analysis: A 5% solution in hexane of each sample was prepared and then injected 0.2 microliter using the split injection technique, then analyzed by GC-MS. The GC system operated in spitless injection mode, and the purge valve was activated 2min after the injection. A 30m length, 0.25mm diameter and 0.25um film thickness column was used for the cashew oil analysis with the following temperature program, oven temperature programmed at 60 ^{o} C, immediately temperature programmed increases at 3 ^{o}C/min to 240 ^{o}C Helium was used as the carries gas at constant flow rate of 1.5ml/min with vacuum compensation. The GC injection temperature was maintained at 290 ^{o} C. The temperature of the MS ion source and transfer line was kept at 250 ^{o}C and 300^{o}C, respectively, sample extract or standard solution was manually injected with a solvent delay at 6min, the MS spectra is between 50 – 300 AMU. The Mass Spectrometer was operated with electron impact ionization (E.I) mode with electron energy of 70cv (Pripdeevech *et al*, 2010). Software

was used for instrument parameters optimization as well as the data acquisition and analysis.

RESULTS AND DISCUSSION

Physicochemical parameters of oils extracted for different parts of two varieties of Anacardium occidentale: The results in Table.1; shows the relative densities and saponification values of oils extracted using two different methods from different parts of the two varieties of *Anacardium occidentale* The relative density of cashew nut shell oil extracted from the two varieties, red and yellow from soxhlet method had the highest with a value of 0.9460 g/cm^2 and 0.8920 g/cm^2 for steam methods of extraction, followed by oil extracts from cashew root bark 0.8890 and 0.8900 of red and yellow varieties respectively.

Table 1: Relative Density of oils extracts at T/25[g/cm^2] and Saponification Values of Cashew Plant Oil Extracted from difference Parts [mg.KOH/g]

		Relative Density		Saponification Values	
Cashew plant oil extract	**Varieties**	**Soxhlet Extraction**	**Steam Distillation**	**Soxhlet Extraction**	**Steam Distillation**
Cashew Nut	Red	0.9460	0.8920	137.50	133.05
Shell oil	Yellow	0.9460	0.8920	140.01	130.06
Cashew Stem	Red	0.8725	0.8689	132.05	128.25
Bark Oil	Yellow	0.8724	0.8723	133.11	128.21
Cashew Leaf	Red	0.8455	0.8450	126.18	122.00
Oil	Yellow	0.8480	0.8480	125.15	125.06
Cashew Root	Red	0.8890	0.8887	131.75	128.00
Bark Oil	Yellow	0.8900	0.8890	133.50	129.20
Average		**0.8887**	**0.8745**	**132.45**	**127.98**

The lowest relative density of the oil extract was found in cashew leaf oil with value of 0.8455 and 0.8480 for red and yellow varieties respectively. The average relative density of oils extracted through Soxhlet extraction method was slightly higher than those extracted by steam distillation method, which shows that cashew plant oils extracted through steam distillation method are lighter in volume, that is p = m/v where p = density, m = mass, v = volume' This means that oils extracted through steam distillation method had slight reduction in mass per volume. From table 1; cashew nut shell oil had the highest value of saponification value for red variety 137.50 mgKOH/g and yellow variety 140.01mgKOH/g and the lowest value was found in leaf 126.18 and 125.15 for red and yellow variety respectively. These values were similar with the result of Sekunowo and Uduh (2022). The higher the saponification value, the more capable the oil is in making soap. So, the oils will not be good in soap making.

Table 2 shows the Iodine Values (mg iodine/100g]) and Refractive index extracted using two different methods from different parts of the two varieties of *Anacardium occidentale* for Soxhlet extraction, the iodine value of cashew nut shell oil had the highest 35.00 mg iodine/100g and 33.00 mg iodine/100g for red and yellow varieties respectively and the leaf oil had the lowest iodine value 20.00mgiodine/100g and 21.00 mg iodine/100g for red and yellow varieties respectively.

Table 2: Iodine Values (mg iodine/100g]) and Refractive index of Oil Extracted from Different Parts of two varieties of cashew plant

		Iodine Values mg iodine/100g]		Refractive index	
Cashew plant oil extract	**Varieties**	**Soxhlet Extraction**	**Steam Distillation**	**Soxhlet Extraction**	**Steam Distillation**
Cashew Nut Shell	Red	35.00	30.00	1.8	1.7
oil	Yellow	33.00	30.00	1.7	1.65
Cashew Stem	Red	26.00	20.00	1.4	1,40
Bark Oil	Yellow	24.00	20.00	1.39	1.39
Cashew Leaf Oil	Red	20.00	19.00	1,54	1.45
	Yellow	21.00	22.00	1.54	1.44
Cashew Root Bark	Red	24.50	22.40	1.52	1.50
Oil	Yellow	25.00	23.00	1.55	1.50

As determined, the iodine value of cashew nut shell oil showed a higher degree of unsaturation than oils from the leaf, stem bark and root bark. Oils extracted by soxhlet method gives more value of iodine than oils extracted by steam distillation. From the table, the iodine value in all the extracts ranges from 19.00 mg iodine/100g to 35.00 mg iodine/100g, which is within the range of some values reported in literatures in the same food (Eliah, 2015; Abitogu and Borokini, 2010). In Soxhlet extraction (Table 2), the oils extracted from cashew nut shell had refractive index of 1.8 and 1.7 for red and yellow varieties respectively shows higher

refractive index them oils from other parts of the tree. In comparison for Soxhlet extraction and steam distillation for each oil, oils extracted through Soxhlet extraction method is slightly higher in refractive index value than those extracted through steam distillation method except in the case of cashew leaf oil. This may be loss of some components during steam distillation process for other parts other than leaf oil. Table 3; shows the result of acid values (mgKOH/g) and Peroxide values [MMOL/L of oils extracted using the two different methods from different parts of the two varieties of *Anacardium occidentale* Table 3; shows that the highest acid value was found in red variety of cashew nut shell oil, 12.50 mgKOH/g and 13.00 mgKOH/g for soxhlet extraction and steam distillation respectively.

Table 3: Acid values (mgKOH/g) and Peroxide values (mmol/l) of oil extracted from different parts of different parts of two varieties of cashew plant

		Acid values tree (mgKOH/g)		**Peroxide values (mmol/l)**	
Cashew plant oil extract	**Varieties**	**Soxhlet Extraction**	**Steam Distillation**	**Soxhlet Extraction**	**Steam Distillation**
Cashew Nut	Red	12.50	13.00	1.40	1.50
Shell oil	Yellow	10.00	11.00	1.57	1.67
Cashew Stem	Red	11.00	11.00	1.60	1.20
Bark Oil	Yellow	11.50	11.80	1.40	1.45
Cashew Root	Red	12.00	13.00	1.50	1,42
Bark Oil	Yellow	12.30	12.50	1.62	1.62
Cashew Leaf	Red	11.00	11.10	1.20	1.22
Oil	Yellow	11.40	11.40	1.32	1.32

Also, the lowest acid value was found in yellow cashew nut shell for Soxhlet extraction. A good oil, the acid value should be very low. The increase in acid value should be taken as an indicator of oxidation of oil which may lead to gum and sludge formation beside corrosion [Sharma, & Jain, 2015]. Also, oils extracted through steam distillation indicated small increase in acid value to the ones extracted through soxhlet extraction. These results are in line with earlier researcher with cashew nut shell liquid with acid value 11.32 mgKOH/g. These results were found to be within the range reviewed from some Nigeria plant foods (Aremu *et al.*, 2015). The results from Table 3, shows that the oil that had highest value of peroxide 1.67 mmol/l is cashew nut oil extract through steam distillation and lowest 1.20 mmol/l. The peroxide value is used as an indicator of determination of oil condition, fresh oil have value less than 10 mmol/l while values between 20 to 40 mmol/l results to rancid taste. The peroxide values of all the cashew oil extracts from the table ranged from 1.22 to 1.62 mmol/l. The low value indicates that the oil can resist lipolytic hydrolysis and oxidative deterioration (Adebayo *et al* 2012). Also, from the report of Api, *et al* (2015), the peroxide value result of oils from all the parts of cashew tree may except the cashew nut shell oil be graded as fragrance oil. The results of viscosity of oils extracted using the two different methods from different parts of the two varieties of *Anacardium occidentale* is shown in table 4. From the viscosity values obtained in the table 4; the most viscose oil as oils was from yellow cashew nut shell extracted through Soxhlet method with the value 31 mpa.s, followed by 3 0mpa.s value obtained from red cashew nut shell and leaf oil extracted through steam distillation.

Table 4: Viscosity of Oil Extracted from Difference Parts of the Cashew Tree at 25^{o}c [mpa.s]

Plant Oil Extract	**Varieties**	**Color of Oil**	**Soxhlet Extraction**	**Steam Distillation**
Cashew nut Shell	Red	Dark brown	30	30
Oil	Yellow	Dark brown	31	30
Cashew Stem	Red	Light brown	28	29
Bark Oil	Yellow	Brown	28	28
Cashew Root	Red	Brown	28	29
Bark Oil	Yellow	Brown	29	28
Cashew Leaf Oil	Red	Green	30	32
	Yellow	Green	29	30

The average viscosity of oil extracted at 25^{O}C through soxhlet method and steam distillation method were 29.125 mpa.s and 29.50 mpa.s respectively. These results shows that the viscosity of oil extracts from the nut shell, root bark stem bark and leaf were lighter than viscosity of olive oil 34 mpa.s at 40^{O}C, sunflower oil 32.20mpa.s at 38^{O}C and walnut oil 29.60 mpa.s at 38^{0} C (Diamante and Lan, 2014)

.

Table 5: Bioactive compositions of cashew nut shell oil extracted through Soxhlet extraction and steam distillation method (%)

Compounds	Retention time(s)	Soxhlet extraction concentration	Steam distillation concentration	Molecular mass (g/mol)	Molecular formula
Cardol	4.41	23.34	20.34	320.5	$C_{22}H_{35}O_2$
Anacardic acid	7.45	15.40	13.40	342.5	$C_{22}H_{36}O_3$
Cardanol	8.65	17.85	19.30	298.5	$C_{21}H_{30}O$
2-methyl cardol	10.35	12,35	15.65		$R=C15H_{31\_n}$
Triaconten	11.12	6.31	_	436.8	$C_{30}H_{60}O$
B-Sitosterol	14.11	4.5	_	414.7	$C_{26}H_{50}O$

Table 6: Bioactive compositions of cashew stem bark oils extracted through Soxhlet extraction and steam distillation method

S/N	Compounds	Compounds	Retention time	Soxhlet extraction Con.	Steam distillation Con.	Molecular mass (g/mol)	Molecular fomular
1	Alkaloids	Quinine	29	-	8.5	324.4	$C_{20}H_{24}NO_2$
		Morphine	24	4.5	3.5	285.34	$C_{17}H_{19}NO_3$
2	Tannins	Tannic acid	35	8,5	-		
3	Other polyphenol	Polyphenol	40	3.4	3.4	500-3000	
4	Flavonoids	Anthoyanin	21	6.7	11.5	207.25	$C_{15}H1_1O$
		Luteolin	23	-	3.3	286.24	$C_{15}H_{10}O_6$
5	Coumarins	Coumarins	15	7.8	4,7	146.4	$C_9H_6O_2$
6	Terpenoids	Myrtenal	17	8.5	5.6	150.20	$C_{10}H_{14}O$
		Geranoil	19	5.5	5.7	152.25	$C_{10}H_{18}O$
7	Saponins	TrIterpene glycosides	45	10.1	12.5		$-C_{30}H_{48}O_7S$

Table 7: Bioactive composition of cashew root bark oil extracted through soxhlet extraction and steam distillation method.

S/N	Compound group	Compound	Retention time	Soxhlet extraction Con.	Steam distillation Con.	Molecular mass	Molecular formula
1	Alkaloids	Quinine	29	-	6.0	324.4	$C_{20}H_{24}N_2O_2$
		Morphine	24	3.6	11.5	285.4	$C_{17}H_{19}NO_3$
2	Tannins	Tannic acid		8.5	6.6	1701.19	$C_{76}H_{52}O_{46}$
		Polyphenoil	35	3.6	5.8	500-300	
3	Flavonoids	Anthoyanin	21	5.6	8.7	207.25	$C_{15}H_{11}O$
		Luteotin	23	3.5	4.5	286.24	$C_{15}H_{10}O6$
4	Coumarin	Coumarin	15	8.1	3.4	146.4	$C_9H_6O_2$
5	Terpenoids	Myrtenal	17	6.8	7.8	150.20	$C_{10}H_{14}O$
6		Geraniol	19	0.9	8.1	152.25	$C_{10}H_{18}O$
7	Saponins	Triterpene glycoside	40	10.0	11.0		$-C_{30}H_{48}O_7S$

Table 8: Bioactive composition of cashew leaf oils extracted through soxhlet extraction and steam distillation method

S/N	Compound group	Compound	Retention time	Soxhlet extraction Con.	Steam distillation Con.	Molecular mass	Molecular formula
1	Alkaloids	Quinine	29	-	5.0	324.4	$C_{20}H_{24}N_2O_2$
		Morphine	24	3.6	11.5	285.4	$C_{17}H_{19}NO_3$
2	Tannins	Tannic acid		3.5	1.6		
		Polyphenoil	35	3.6	5.8	500-300	
3	Flavonoids	Anthoyanin	21	5.6	6.7	207.25	$C_{15}H_{11}O$
		Luteolin	23	12.5	9.5	286.24	$C_{15}H_{10}O_6$
4	Coumarin	Coumarin	15	8.1	3.4	146.4	$C_9H_6O_2$
5	Terpenoids	Myrtenal	17	6.8	7.8	150.20	$C_{10}H_{14}O$
		Geraniol	19	0.9	8.1	152.25	$C_{10}H_{18}O$
6	Saponins	Triterpene glycoside	40	7.0	7.0		$-C_{30}H_{48}O_7S$

GC-MS Analysis of Composition of Oils Extracted from Cashew Nu,t Shell, Stem Bark, Root Bark and Leaf of Anacardium occidentale: The results of the GC-MS analysis of the different parts of the plants are shown in Tables 5- 8. Table 5 shows the result of GC-MS analysis of cashew nut shell oil extracted using the two different methods from different parts of *Anacardium occidentale. From* this result, soxhlet extraction was more effective in extraction of oils from cashew nut shell than steam distillation method. At the same condition of GC-MS analysis, Soxhlet extraction gave 23.34% cardol, 15.40 % anacardic acid, 17.85% cardol 12.5% 2-methyl cardol, 6.31% triaconten, and 4.5% B-sitsterol while stem distillation gave only

20.34% cardol, 13.40 % anacardic acid, 19.0%caranol and 15.65% 2-methyl cardol. This lesser oil composition level from oils extracted from team distillation may be due to loss of some particles during distillation processes. The bioactive compositions of cashew nut shell oil extracted by Soxhlet extraction and steam distillation methods, most of compositions has been reported by other researchers (Aletor *et al.* 2007).

In this study, cashew stem bark, cashew root bark and cashew leaf oils bioactive compounds identified include alkaloids, tannins, flavonoids, coumarins, tepenoids, saponins, and other polyphenols. Onuh *et al*., (2017) and Ojezele and Ogunbiade, (2013) reported similar compositions of these compounds which varies in each research, which may be as a result of different analytical methods and conditions. These cashew tree parts are rich in wide variety of secondary metabolites which have been found in vitro to have antimicrobial properties. Terpenoids give plants aroma and colours. Quinines and tannins are responsible for plant pigments (Marjorie, 1999). Tannic acid is a specific form of tannin, a weak acid with numerous phenol group in structure. It has antioxidant properties and may promote good health. It is used to treat a varieties of maladies Luteolin is a type of flavonoid with a yellow crystalline appearance with potentials for cancer prevention and therapy defense against predictors (Okoronkwo *et al*, 2020; Venugopala *et al.*, 2013). Morphine is a severe pain reliever and Quinnine is used as anti-malarial drug. Polyphenols are a category of plant compounds that offers health benefits, it boost digestion and brain health as well as protect against heart disease, type 2 diabetes, and even certain cancer. They are antioxidant, meaning they can neutralize harmful free radicals and they reduce inflammation' Anthoyanins are large group of red-blue plant pigments occurring in all developed plants. It possesses antioxidant property and has different other pharmacological properties (Wegdan *et al*., 2020). Saponin has been shown to have beneficial effect by binding cholesterol and ultimately lowering its amount in the body

Conclusion: The oils extracted from the cashew nut shell, the leaf, root bark and stem bark contained biological active compounds that are responsible for their bioactivities. The presence of cardol, Anacadic acid, Cardanol, 2-methylcardol, Triaconten and β-sitosterol in nut shell gives credence for repellent and insecticidal properties. Also, the antimicrobial, antioxidant, repellent and insecticidal properties of the stem bark, root bark and leaf were due to presence of alkaloids, tannins, flavonoids, coumarins, terpenoids and saponins.

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