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## Chemical Characterization of Colorants Isolated from the Fruit Juice of *Anacardium occidentale* (Cashew)

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### Abstract

This study focuses on isolating and characterizing colorant from *Anacardium occidentale* (Cashew) fruit juice. The juice was concentrated and fractionated into n-hexane (NH), methanol/dichloromethane (MeOH/DCM), methanol/ethyl acetate (MeOH/EA) fractions, and a residue. Phytochemical analysis and GC-MS were used to identify bioactive compounds, and the residue was further fractionated into 12 sub-fractions (AORF1–AORF12) and analysed with FTIR and UV-Vis spectroscopic methods. Phenols, alkaloids, flavonoids, glycosides, and terpenoids were present in the MeOH/DCM, MeOH/EA fractions, and residue, with tannins and saponins found only in the residue. Key bioactive compounds, including co-pigments, were identified. The pigments interacted with cellulosic materials, suggesting their role in fabric staining. The study reveals the potential of Cashew fruit juice as a natural pigment source, with possible applications in textile dyeing. Further research is recommended to explore the full scope of *A. occidentale* pigments for industrial applications, including optimization of extraction methods and more in-depth studies on their use as natural dyes in various materials. Additionally, investigating the eco-friendly and sustainable aspects of these pigments in comparison to synthetic dyes is encouraged.

### 1. Introduction

Color is considered to be the primary feature perceived by the senses that represent a crucial role for centuries in the acceptability of finished products to enhance appearance and aesthetic value (Wang & Hsu, 2019). Plant pigments, the impeccable natural source of color, display enormous potential to substitute many of the synthetic colorants (López-Cruz *et al.*, 2023). because natural pigments and colorants are non-toxic and non-mutagenic, have desirable pharmacological properties, and are friendly to the environment (Di Salvo *et al.*, 2023)(Szadkowski *et al.*, 2022). The growing demand for natural pigments is because of their wide range of applications, highlighted in the food industry, cosmetics, and animal feed. Such increased demand has influenced the research for new sources and processes to obtain natural colorants (Gemelli *et al.*, 2024).

The cashew tree, *Anacardium occidentale* L., belongs to the *Anacardiaceae* family of plants, which also includes poison ivy, mangos, and pistachios. The *Anacardiaceae* family comprises 76 genera and about 600 species, arranged into five tribes: *Anacardiaceae*, *Dobineae*, *Rhoaeae*, *Semecarpeae*, and *Spondiadeae*. *Anacardium occidentale*, the cashew tree, is indigenous to Brazil and the Lower Amazon. Drought and bad soil are only two of the many situations that the cashew tree can withstand. (Kunjumon *et al.*, 2022). Cashew has a wide range of secondary metabolites, according to phytochemical research. Phytochemical substances such as triterpenoids, phenolics, and volatile oils can be found in the ethanolic extract of Cashew nuts. Phenolics, volatile oils, xanthoprotein, and

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carbohydrates were all present in ethylacetate extract. Triterpenoids, phenolics, volatile oils, flavonoids, xanthoprotein, and carbohydrates were all found in acetone extract. Anthocyanins, carotenoids, ascorbic acid (vitamin C), flavonoids, and other polyphenols, as well as mineral components, are abundant in this tree. The bark and leaves are employed In production of folk medicine (Kunjumon, 2022).

This research study aimed to extract, isolate and characterize the pigments responsible for stain on fabrics from cashew apple fruit juice and ascertain their chemical nature.

## 2. Material and Methods

### 2.1 Sample Collection and Identification

Ripe and fresh cashew fruits were collected from Alapa, Asa Local Government Area of Kwara State. The plant material was then authenticated at the Herbarium section of the Department of Plant Biology, University of Ilorin, where a Voucher Specimen with Number UILH/001/970/2023 was deposited.



Figure 1: Cashew (*Anacardium occidentale*) Fruits

### 2.2 Preparation of the Plant Sample

The Authenticated *Anacardium occidentale* (cashew) fruits were rinsed with Tap water and then Distilled water. The nuts were dislodged manually. Afterwards, allowing the water to drain and dry off the cashew apple fruit.

### 2.3 Extraction and Concentration of the Cashew Fruits Juice

The cashew fruit juice was extracted from the cashew apple (8.85 kg) using locally made fruits juice extractors. A five (5) liters cashew fruits juice was obtained equivalent to 5.18 kg in weight. The aqueous fruit juice was concentrated by complementary use of regulated water bath and subsequently freeze-drying. The fruit juice after concentration was reduced to 212 ml equivalent to 149.38 grams.

### 2.4 Partitioning of Chemical Components of the Fruit Juice

The concentrated aqueous cashew fruit juice was reconstituted in methanol and placed in the separating funnel and partitioned into n-hexane fraction. The process was repeated with binary solvent mixture of methanol (MeOH) and dichloromethane (DCM) to obtain MeOH/DCM fraction. Also, binary solvent mixture of methanol (MeOH) and ethyl acetate (EA) to obtain MeOH/EA fraction. This affords four fractions, the n-hexane, MeOH/DCM, MeOH/EA and the residue, which were concentrated and kept in labelled sample bottles.

### 2.5 Column Chromatography Technique

The residue of the cashew fruit juice was further fractionated using gravity column chromatography. Fifty grams (50 g) of the extract was loaded on a column (73 cm of length and 3.2 cm diameter) with

silica gel (70-230 plus 240 – 300 mesh size, Merck, Germany) as the stationary phase. The loaded column was eluted with n-hexane, dichloromethane, ethyl acetate and methanol in a mixture of an increasing polarity. Forty-six eluent of 200 ml each were collected these were pool together based on their thin layer chromatography (TLC) profile to yield twelve fractions (AO<sub>R</sub>F1–AO<sub>R</sub>F12).

## 2.6 Phytochemical Screening of the extracts

The MeOH/DCM, MeOH/EA fractions and the residue were subjected to qualitative phytochemical analysis for the presence of tannins, saponin, flavonoids, alkaloids, steroids, glycosides and terpenoids using standard procedures as described (Mani *et al.*, 2015)

## 2.7 Characterization of fractions from *Anacardium occidentale* fruit juice

### Ultraviolet- visible (UV-Vis) spectroscopy

The UV- Visible analysis of all the column chromatographic fractions of *Anacardium occidentale* fruit aqueous extract were carried out using the Aquamate V4.60 UV/Visible spectrophotometer at the Department of Chemistry, University of Ilorin, Ilorin, Nigeria.

### Infrared (IR) spectroscopy

The infrared spectra of all the column chromatographic fractions of the *Anacardium occidentale* fruit aqueous extract were determined using the Thermo Scientific NICOLE iS5 FTIR spectrometer at the Department of Chemistry, University of Ilorin, Nigeria.

## 3. Results and Discussion

### 3.1 Phytochemical Screening

The Phytochemical analysis revealed the presence of alkaloids, glycosides, terpenoids, flavonoids and phenols and reducing sugar in the MeOH/DCM, MeOH/EA, however saponins and tannins are also present in the residue of *Anacardium occidentale* fruit juice but no reducing sugar (table 1)

**Table 1: Result of phytochemical screening of MeOH/DCM, MeOH/EA and the residue**

Phytochemicals	MeOH/DCM	MeOH/EA	Residue
Alkaloids	+	+	+
Saponins	-	-	+
Phenol	+	+	+
Tannins	-	-	+
Flavonoids	+	+	+
Steroids	+	+	+
Terpenoids	+	+	+
Glycoside	+	+	+
Reducing sugar	+	+	-

Ngozika *et al.*, 2020, also reported the presence of alkaloids; tannins; glycosides; flavonoids and phenols in both ethanol and aqueous extracts of *A. occidentale* dried fruit. In addition, (Ndiaye *et al.*, 2022) reported the presence of reducing sugars in the fruit juice of *A. occidentale*

### 3.2 Ultraviolet -visible (uv-vis) spectroscopic analysis of the isolated pigments

**Table 2: interpretation of the UV-visible Spectra (figure 2) of the isolated pigments**

EQ Column chromatographic FRACTION (F)	Wavelength (nm)	Absorbance	Possible Electronic Transitions (Mabasa <i>et al.</i> , 2021)
AO <sub>R</sub> F9	236	4.246892	$\pi-\pi^*$
	280	4.499977	$\pi-\pi^*$
	383	2.230527	$n-\pi^*$
AO <sub>R</sub> F10	235	4.238972	$\pi-\pi^*$
	299	4.462711	$\pi-\pi^*$
	355	1.556459	$n-\pi^*$
AO <sub>R</sub> F11	244	4.22227	$\pi-\pi^*$
	287	4.476641	$\pi-\pi^*$
	358	1.647029	$n-\pi^*$
AO <sub>R</sub> F12	237	3.595862	$\pi-\pi^*$
	291	4.155813	$\pi-\pi^*$
	359	1.430756	$n-\pi^*$

The UV-visible spectroscopy analysis results obtained showed that the isolated pigments have Three absorption bands around (300-390 nm) and (240-280 nm). This shows the presence of chromophores and other unsaturation in the isolated pigments (Butnariu, 2023). The bands were attributed to  $\pi-\pi^*$  transition of the aromatic rings and  $n-\pi^*$  transition of the C=O group (Kasal *et al.*, 2010). Absorption spectroscopy is an essential tool for quantitation of flavonoids isolated from plant extracts. The absorption spectra of flavonoids typically consist of two major bands, band I (300–380 nm) and band II (240–295 nm), where the former engenders a yellow color of the isolated pigments (Taniguchi *et al.*, 2023). Sometimes an additional maximum is possible, Band III, around 330 nm (Butnariu, 2023). The UV-VIS range for Tanin in solution depicts two bands at 213 and 276 nm, both assigned for  $\pi\rightarrow\pi^*$  transitions given by aromatic units and C=O groups in UV VIS-region as reported by (Anburaj & Jothiprakasam, 2017). The chromophores present in the isolated pigments were revealed by spectra of three peaks as shown below. The chromophore displayed by the first peak at  $\lambda_{\max}$  239, 246, 241 and 239 nm of AO<sub>R</sub>F9, AO<sub>R</sub>F10, AO<sub>R</sub>F11, and AO<sub>R</sub>F12 respectively can be identified as C=C due to delocalized electron from fluorescent aromatic compounds (benzene rings). The transition of electrons from highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbital (LUMO) is commonly expressed as  $\pi-\pi^*$ . This can be explained by high energy gap and short wavelength which is characteristic of C=C chromophore (Kimutai Bernard *et al.*, 2019). The second peak absorbed at  $\lambda_{\max}$  383, 355, 358 and 359 nm was due to non-bonding electron or lone pairs electron on the oxygen atom (carbon oxygen double bond or C=O) which is characterized by absorption of UV-VIS light at a lower frequency, lower energy and higher wavelength as a consequence of electron jump from non-bonding orbital to anti bonding orbital commonly referred as  $n\rightarrow\pi^*$  electron jump which is in agreement with (Kimutai Bernard *et al.*, 2019). The pigments isolated showed additional absorbance at 280, 299, 287, and 291 nm that occurs at higher wavelength than benzene with  $\lambda_{\max}$  255 nm, meaning that a functional group attached to benzene ring is responsible for bathochromic shift (Kimutai Bernard *et al.*, 2019) (Butnariu, 2023), as shown by the absorption values (table).

### 3.3 Fourier-transform infrared spectroscopic analysis of the isolated pigments

**Table 3: interpretation of the FTIR Spectra of the Isolated Pigments**

Functional groups →	O-H Stretch (cm <sup>-1</sup> )	C-H Stretch (cm <sup>-1</sup> )	C≡C Stretch (cm <sup>-1</sup> )	C=O Stretch (cm <sup>-1</sup> )	C=C Stretch (cm <sup>-1</sup> )	C-O-C Stretch (cm <sup>-1</sup> )	O-H Bend (cm <sup>-1</sup> )	C-O Stretch (cm <sup>-1</sup> )	Out of plane C-H stretch (cm <sup>-1</sup> )
Isolated Pigments↓									
AO F <sub>R</sub> 9	3265	ND	2124.6	ND	1640 1416	1267	ND	10134	700
AO F <sub>R</sub> 10	3250	2943	2117.1	ND	1640 1416	1260	1349.3	1036	800
AO F <sub>R</sub> 11	3362	2945 2833	ND	1722	1639 1446	1252	1379.1	1103 1021	725
AO F <sub>R</sub> 12	3355	2952 2840	ND	ND	1655 1526 1420	1225	ND	1116 1020	700

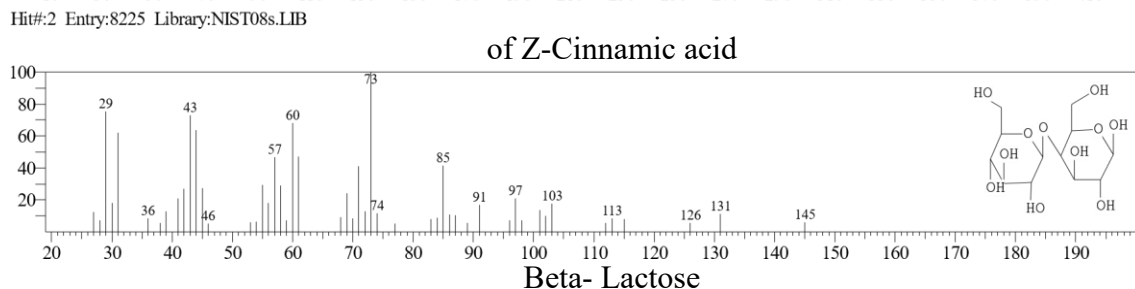
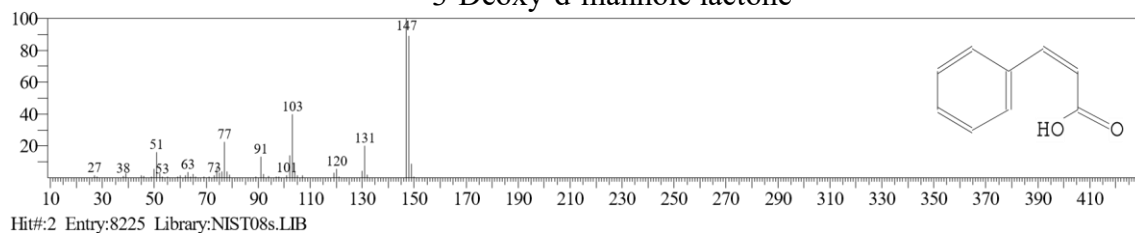
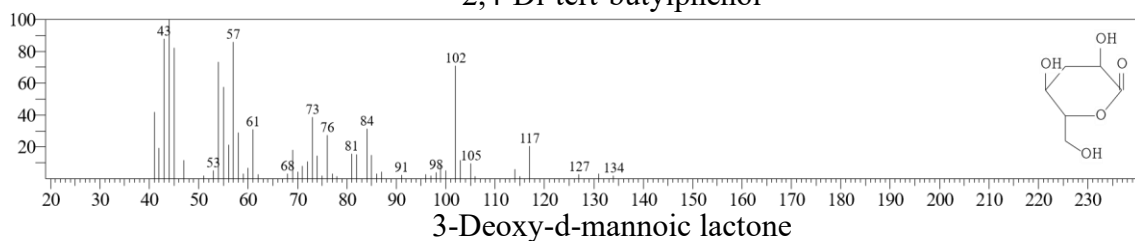
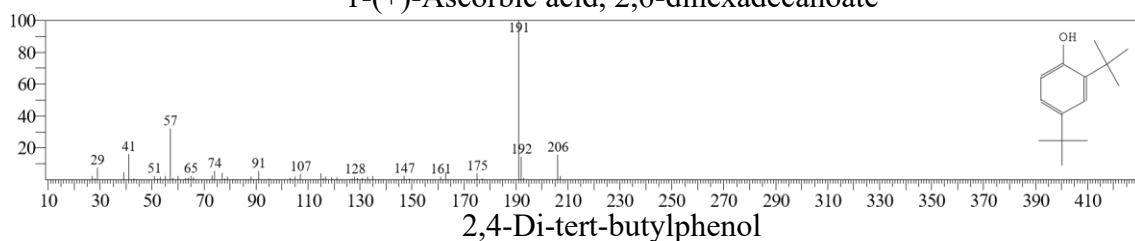
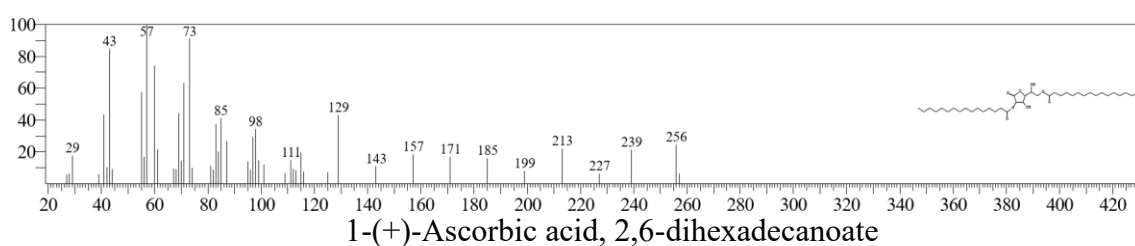
**Keys:** AO F<sub>R</sub> = *Anacardium Occidentale* Residue Fraction; ND = Not Detected

Further characterization of the isolated pigments was looked through the functional groups using FTIR analysis (Fig. 3) which had a band at 3265, 3250, 3362 and 3355 cm<sup>-1</sup> indicating the presence of O–H stretching (Bekele *et al.*, 2024), the bands displayed at frequency 2943, 2945, 2833, 2952 and 2840 cm<sup>-1</sup> vibrations is due to C-H stretch (Kimutai Bernard *et al.*, 2019). Hydrolysable tannins exhibit a peak around 1722 cm<sup>-1</sup> for carbonyl (C=O) stretching (Ruiz-Aquino *et al.*, 2023). The combination of bands at 2125, 1640 and 2117 cm<sup>-1</sup>, 1640 cm<sup>-1</sup> in the spectra of AO F<sub>R</sub>9 and AORF10 respectively is considered as indicative of conjugated C=C bonds (Gemelli *et al.*, 2024). Additionally, the bands at 1640 and 1416 cm<sup>-1</sup> correspond to the stretching vibration of the C=C aromatic ring (Swier *et al.*, 2018b). The absorption band at frequency 1349 cm<sup>-1</sup> and 1379 cm<sup>-1</sup> is as a result of the O-H of either a phenolic or carboxylic group (Kimutai Bernard *et al.*, 2019). The 1267, 1260, 1252 and 1225 cm<sup>-1</sup> absorption bands is due to C-O-C of the pyran rings (Falcao *et al.*, 2018), while the absorption band between 1013.8, 1036.2, 1021 and 1020 cm<sup>-1</sup> is due to C-OH of phenolic compound that is out of plane deformation which is agreeing with (Kimutai Bernard *et al.*, 2019).

The FTIR profile for the isolated pigment AO F<sub>R</sub>9 shows bands at 3265 cm<sup>-1</sup>, 2125 cm<sup>-1</sup>, 1640 cm<sup>-1</sup>, 1416 cm<sup>-1</sup>, and around 590 cm<sup>-1</sup>, while AO F<sub>R</sub>10 show bands at 3250, 2117, 1640, 1416, and also around 590 cm<sup>-1</sup> confirmed the distinguish characteristics of anthocyanins in agreement with (Neciosup-Puican *et al.*, 2024) (Jeyaram & Geethakrishnan, 2020) (Swier *et al.*, 2018a). FTIR analysis of AO F<sub>R</sub>11 revealed various functional groups, such as O-H, C=O, C=C, C-O-C and C-OH (ethers, alcohols, and sugars), and C-H which confirmed the presence of hydrolysable tannin (Gallotannin) in accordance with previous work by (Zhang *et al.*, 2017)(Ruiz-Aquino *et al.*, 2023) (Wahyono *et al.*, 2019); (Falcao *et al.*, 2018); (Mahmoud *et al.*, 2015); (Eldin *et al.*, 2016), while the FTIR spectrum of AO F<sub>R</sub>12 confirmed the existence of functional groups which were reported by findings as the distinguishing characteristics of condensed Tannins (Marques *et al.*, 2021); (Zhang *et al.*, 2017)(Falcao *et al.*, 2018).

### 3.4 GC–MS analysis of n- hexane, MeOH/DCM, MeOH/EA Extracts

The GC–MS analysis of n-hexane, MeOH/DCM, MeOH/EA extracts does not reveal any compound reported as colorant in previous literatures, however indicated the presence of various bioactive compounds, among which are molecules that have been discovered as co-pigments (Fig. 3). Phenolics, amino acids, alkaloids, and organic acids are the most common co-pigment compounds. Co-pigments are generally colorless molecules that exist naturally in plant cells alongside anthocyanin (Gençdağ *et al.*, 2022). Co-pigmentation is considered an effective way to stabilize anthocyanins against adverse environmental conditions. This phenomenon is commonly reserved for the non-covalent interaction between anthocyanins and colorless organic molecules (co-pigment) (Tang *et al.*, 2023). These interactions include both hyperchromic shifts, which darken as absorbance increases, and bathochromic shifts, which positive increase the wavelength at maximum absorbance ( $\lambda_{max}$ ) (Gençdağ *et al.*, 2022).



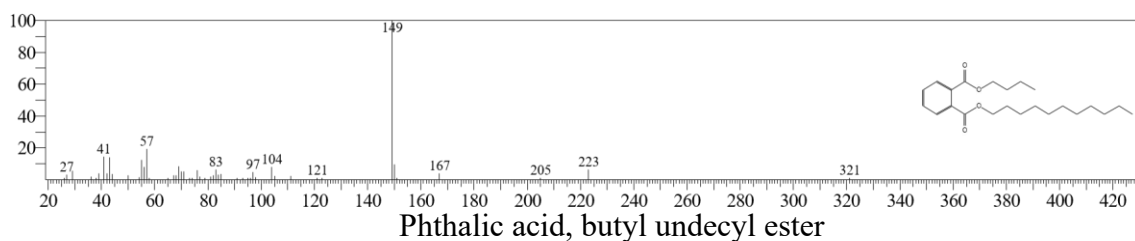


Figure 4: GC-MS Spectra of co-pigments in cashew fruit juice

**Table 4: GC-MS analysis of n-hexane Extract**

S/N	COMPOUND	Molecular formular	R. Time(min)	% area	Molecular weight
1	1-Dodecanol	(C <sub>12</sub> H <sub>26</sub> O)	6.450	0.28	186
2	Dodecane	(C <sub>12</sub> H <sub>26</sub> )	6.535	1.39	170
3	5-Hydrxoymethylfurfural	(C <sub>6</sub> H <sub>6</sub> O <sub>3</sub> )	6.881	1.65	126
4	1-Tridecene	(C <sub>13</sub> H <sub>26</sub> )	7.316	0.25	182
5	4, 7- Dimethyl undecane	(C <sub>13</sub> H <sub>28</sub> )	7.556	0.28	184
6	1-Tetradecane	(C <sub>14</sub> H <sub>28</sub> )	8.453	2.01	196
7	Tetradecane	(C <sub>14</sub> H <sub>30</sub> )	8.525	2.97	198
8	Z-Cinnamic acid	(C <sub>9</sub> H <sub>8</sub> O <sub>2</sub> )	8.946	8.85	148
9	9-Methylnonadecane	(C <sub>20</sub> H <sub>42</sub> )	9.107	0.88	282
10	Eicosane	(C <sub>20</sub> H <sub>42</sub> )	21.326	1.42	282
11	2,4-Di-tert-butylphenol	(C <sub>14</sub> H <sub>22</sub> O)	9.601	0.43	206
12	Dodecanoic acid	(C <sub>12</sub> H <sub>24</sub> O <sub>2</sub> )	9.978	0.42	200
13	1-Nonadecene	(C <sub>19</sub> H <sub>38</sub> )	35.397	21.69	266
14	Hexadecane	(C <sub>16</sub> H <sub>34</sub> )	10.299	2.73	266
15	Chloroacetic acid, 4-hexadecyl ester	(C <sub>18</sub> H <sub>35</sub> ClO <sub>2</sub> )	10.361	0.71	318
16	Sulfurous acid, 2-propyltetradecyl ester	(C <sub>17</sub> H <sub>36</sub> O <sub>3</sub> S)	10.817	0.33	320
17	Cis -1-Chloro-9-octadecene	C <sub>18</sub> H <sub>35</sub> Cl	11.449	1.45	286
18	(Z) -7- Hexadecenal	(C <sub>16</sub> H <sub>30</sub> O)	11.525	1.84	238
19	Tetradecanoic acid	(C <sub>14</sub> H <sub>28</sub> O <sub>2</sub> )	11.599	1.39	228
20	Methyl (2-Phenyl-1,3-dioxolan-4-yl stearate	(C <sub>28</sub> H <sub>46</sub> O <sub>4</sub> )	12.025	0.41	446
21	Dodecenyyl succinic anhydride	(C <sub>16</sub> H <sub>26</sub> O <sub>3</sub> )	12.089	0.86	266
22	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy	(C <sub>30</sub> H <sub>52</sub> O <sub>2</sub> )	12.208	0.27	444
23	9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione	(C <sub>11</sub> H <sub>16</sub> O <sub>4</sub> )	12.349	1.22	212
24	iso-propyl 11,12 -methylene-octadecanoate	(C <sub>22</sub> H <sub>42</sub> O <sub>2</sub> )	12.504	1.68	338
25	Methyl 13-methylpentadecanoate	(C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> )	12.829	1.46	270
26	(Z) -13-Docosenamide	(C <sub>22</sub> H <sub>43</sub> NO)	12.886	1.79	337
27	2-Hydroxycyclopentadecanone	C <sub>15</sub> H <sub>28</sub> O <sub>2</sub>	12.962	3.73	240
28	-(+)- Ascorbic Acid 2,6-dihexadecanoate	(C <sub>38</sub> H <sub>68</sub> O <sub>8</sub> )	13.169	30.77	652
29	Ethyl iso-allocholate	(C <sub>26</sub> H <sub>44</sub> O <sub>5</sub> )	13.415	1.80	436
30	3-acetoxy, 7,8-Epoxylanostan-11-ol	C <sub>32</sub> H <sub>54</sub> O <sub>4</sub>	13.510	3.25	502
31	Oleic acid	(C <sub>18</sub> H <sub>34</sub> O <sub>2</sub> )	13.709	0.59	282
32	E-8-Methyl-9-tetradecen-1-ol acetate	(C <sub>17</sub> H <sub>32</sub> O <sub>2</sub> )	13.786	0.73	268
33	1- Pentacosanol	(C <sub>25</sub> H <sub>52</sub> O)	13.939	0.44	368

**Table 5: GC-MS analysis of MeOH/DCM Extract**

S/NO	Compound	Molecular formular	R. time (min)	%Area	Molecular weight
1	(S)-2-Hydroxypropanoic acid	(C <sub>3</sub> H <sub>6</sub> O <sub>3</sub> )	3.208	4.44	90
2	N-(3-Butenyl)-N-methylcyclohexanamine	(C <sub>11</sub> H <sub>21</sub> N)	5.453	8.72	167
3	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	(C <sub>6</sub> H <sub>8</sub> O <sub>4</sub> )	6.102	16.62	144
4	Isosorbide Dinitrate	(C <sub>6</sub> H <sub>8</sub> N <sub>2</sub> O <sub>8</sub> )	6.626	4.28	236
5	5-hydroxymethylfurfural	(C <sub>6</sub> H <sub>6</sub> O <sub>3</sub> )	6.988	23.47	126
6	Methyl 3-hydroxyhexadecanoate	C <sub>17</sub> H <sub>34</sub> O <sub>3</sub>	7.176	8.98	286
7	[5-(Dimethoxy methyl)-2-furyl] methanol	(C <sub>8</sub> H <sub>12</sub> O <sub>4</sub> )	7.584	3.44	172
8	D-Maltose	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	7.898	1.89	342
10	Propyl pentanoate	(C <sub>8</sub> H <sub>16</sub> O <sub>2</sub> )	8.229	2.00	144
11	Alpha-D-Glucopyranside, O-alpha-D-glucopyranosyl-(1.fwdarw.3)-beta-D-fructofuranosyl	(C <sub>18</sub> H <sub>32</sub> O <sub>16</sub> )	8.479	5.81	504
12	Acetic acid, 2-propyltetrahydropyran-3-yl ester	(C <sub>10</sub> H <sub>18</sub> O <sub>3</sub> )	8.632	1.55	186
13	Cinnamic acid	(C <sub>9</sub> H <sub>8</sub> O <sub>2</sub> )	8.907	3.30	148
14	Succinic acid, 2-chlorocyclohexylmethyl ethyl ester	(C <sub>13</sub> H <sub>21</sub> C <sub>1</sub> O <sub>4</sub> )	9.367	0.64	276
15	2-[2-(2,4-dimethoxyphenyl)ethyl]-2,4-dihydro-5-methyl 3H-Pyrazol-3-one,	(C <sub>14</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub> )	9.425	0.48	262
16	Heptose	(C <sub>7</sub> H <sub>14</sub> O <sub>7</sub> )	10.352	0.36	210
17	3-Deoxy-d-mannonic lactone	(C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> )	11.109	1.77	162
18	9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione	(C <sub>11</sub> H <sub>16</sub> O <sub>4</sub> )	12.343	1.61	212
19	Phthalic acid, butyl undecyl ester	(C <sub>23</sub> H <sub>36</sub> O <sub>4</sub> )	12.497	1.81	376
20	1-(+)-Ascorbic acid, 2,6-dihexadecanoate	(C <sub>38</sub> H <sub>68</sub> O <sub>8</sub> )	13.072	2.48	652
21	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[[(trimethylsilyl)oxy]methyl]ethyl] ester,	(C <sub>27</sub> H <sub>52</sub> O <sub>4</sub> Si <sub>2</sub> )	13.171	0.29	496
22	Ethyl iso-allocholate	(C <sub>26</sub> H <sub>44</sub> O <sub>5</sub> )	64.533	5.65	436



**Table 6: GC-MS result of MeOH/EA Extract**

S/NO	Compound	Molecular formular	R. time (min)	%Area	Molecular weight
1	2-Nonanol	(C <sub>7</sub> H <sub>16</sub> O)	3.168	0.93	144
2	N-(3-Butenyl)-N-methylcyclohexanamine	(C <sub>11</sub> H <sub>21</sub> N)	5.475	13.25	167
3	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	(C <sub>6</sub> H <sub>8</sub> O <sub>4</sub> )	6.156	17.60	144
4	5-Hydrxoymethylfurfural	(C <sub>6</sub> H <sub>6</sub> O <sub>3</sub> )	7.014	19.34	126
5	Methyl 3-hydroxyhexadecanoate	(C <sub>9</sub> H <sub>18</sub> O <sub>3</sub> )	7.225	7.45	174
6	Stevioside	(C <sub>38</sub> H <sub>60</sub> O <sub>18</sub> )	15.754	10.57	804
7	3-Deoxglucose	(C <sub>6</sub> H <sub>12</sub> O <sub>5</sub> )	8.307	3.13	164
8	3,4-Anhydro-d-galactosan	(C <sub>6</sub> H <sub>8</sub> O <sub>4</sub> )	8.717	1.88	144
9	3-Deoxy-d-mannoic lactone	(C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> )	44.796	15.64	162
10	Alpha-D-Glucopyranoside, O-alpha-D-glucopyranosyl-(1.fwdraw.3)-beta-D-fructofuranosyl	(C <sub>18</sub> H <sub>32</sub> O <sub>16</sub> )	31.238	6.44	504
11	9,9-Dimethoxybicyclo [3.3.1] nona 2,4-dione	(C <sub>11</sub> H <sub>16</sub> O <sub>4</sub> )	24.147	2.98	212
12	3-hydroxydodecanoic acid	(C <sub>12</sub> H <sub>24</sub> O <sub>3</sub> )	12.975	0.80	216

#### 4. Conclusion

In the present study, the Phytochemical analysis of the residue confirmed that the residue contains bioactive phytochemicals like tannins, phenols, and flavonoids. Further, fractionation of the residue into 12 fractions (AO<sub>R1</sub>-AO<sub>R12</sub>) through column chromatographic technique. FTIR and UV absorbance characterization techniques proved flavonoids and tannins to be the potential pigments in the cashew fruits juice. AO<sub>R</sub> 9 - AO<sub>R</sub> 12 represent the various flavonoids (anthocyanins) and tannins isolated. The interactions of AO<sub>R</sub> 9 - AO<sub>R</sub> 12 with one another and with other compounds (co-pigments) revealed by GCMS analysis of n-hexane (NH), methanol/dichloromethane (MeOH/DCM), methanol/ethyl acetate (MeOH/EA) soluble fractions may be responsible for the formation of the permanent stains on fabrics by the cashew apple fruit juice.

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