

# Phytochemical Profile and Bioactive Compounds in Aqueous Leaf Extract of *Anacardium occidentale*: A GC-MS Analysis

## Abstract

This study investigates the phytochemical composition of the aqueous leaf extract of *Anacardium occidentale* (cashew tree) using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The aqueous extraction yielded a crude leaf extract rich in bioactive compounds. Major compounds identified include  $\gamma$ -Terpinene (13.62%), known for its antioxidant and antibacterial properties, and Securinine (2.08%), which exhibits anticancer and antiviral effects. Additionally, dl- $\alpha$ -Tocopherol (5.6%), a potent antioxidant, was found. Other notable compounds, such as 1,2,3-Benzenetriol (6.53%), demonstrated strong antioxidant and anti-inflammatory properties, while Naphthalene (1.78%) exhibited antimicrobial activity. These compounds, among others identified in the extract, suggest that *Anacardium occidentale* leaves have a rich phytochemical profile with significant medicinal properties. The results underscore the plant's potential for treating oxidative stress-related diseases such as diabetes, cancer, and bacterial infections. This research highlights the therapeutic promise of *Anacardium occidentale* and emphasizes the need for further studies on the synergistic effects of these compounds. These findings could serve as a foundation for developing new, plant-based therapeutic agents for managing chronic diseases and infections.

Key words: *Anacardium occidentale*, GCMS, Phytochemical compounds, Aqueous extracts

## Introduction

*Anacardium occidentale*, commonly known as the cashew tree, is traditionally used in folk medicine across various cultures to treat ailments such as diabetes, hypertension, and microbial infections (Ojewole, 2003; Keshinro & Ketiku, 2009). Its phytochemical properties have gained attention due to the potential therapeutic benefits of its bioactive compounds (Gupta et al., 2005). Phytochemical profiling through advanced techniques like Gas Chromatography-Mass Spectrometry (GC-MS) enables the identification of these compounds, contributing significantly to natural product drug discovery (Brand-Williams et al., 1995; Patel & Kumar, 2015).

## Materials and Methods

Fresh leaves of *Anacardium occidentale* were collected from Ogoni land, Rivers State, Nigeria, and processed for extraction following standard protocols (Kamtchouing et al., 2004). Phytochemical analysis was conducted using GC-MS, allowing for the identification of compounds through comparison with the NIST library (Ahn et al., 2004).

- **Collection and Drying of Plant Material:** Fresh leaves of *Anacardium occidentale* are collected and thoroughly washed to remove any dirt. The leaves are then air-dried in a shaded area for about two to four weeks to avoid degradation of phytochemicals due to sunlight (Ojewole, 2003; Keshinro & Ketiku, 2009).

- **Pulverization:** Once completely dried, the leaves are pulverized into a fine powder using a mechanical grinder to increase the surface area for extraction (Kamtchouing et al., 2004).
- **Aqueous Extraction (Maceration):** The powdered leaves are macerated by soaking in distilled water (or another suitable solvent) at room temperature. The plant material is often left for 24 to 72 hours, depending on the desired extraction efficiency. This allows the bioactive compounds to dissolve into the solvent (Akinmoladun et al., 2010).
- **Filtration:** The extract is then filtered using muslin cloth or Whatman filter paper to remove any plant debris and obtain a clear liquid extract (Ahn et al., 2004).
- **Concentration:** The filtered aqueous extract is concentrated using a rotary evaporator at a low temperature and reduced pressure to avoid thermal degradation of the phytochemicals. This step removes excess water, leaving a concentrated crude extract (Kamtchouing et al., 2004).
- **Storage:** The concentrated extract is stored in airtight containers and refrigerated until further use (Ojewole, 2003).

### Phytochemical Composition

Key bioactive compounds identified include  $\gamma$ -Terpinene, known for antioxidant and anti-inflammatory properties (Ahn et al., 2004; Lee & Lee, 2009), Securinine with anticancer potential (Thomas & Stevenson, 2006), and dl- $\alpha$ -Tocopherol, which acts as a potent antioxidant (Simic, 2000). Furthermore, 1,2,3-Benzenetriol demonstrated strong antioxidant activities (Shao et al., 2017), and Naphthalene was recognized for its antimicrobial properties (Huang & Lee, 2011).

### Results

**Table 1. Key bioactive compounds**

RT (min)	Name of Compound	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)	Biochemical Relevance
2.508	Methylene chloride	CH <sub>2</sub> Cl <sub>2</sub>	84.933	0.41	A solvent, known for environmental toxicity and industrial relevance. Limited direct biochemical relevance.
2.891	Diphenylcarbazone	C <sub>13</sub> H <sub>12</sub> N <sub>4</sub> O	240.26	0.48	Used in analytical chemistry as a metal indicator. Can have antioxidant effects.

3.211	$\gamma$ -Terpinene	C <sub>10</sub> H <sub>16</sub>	136.234	13.62	Known for its antioxidant and anti-inflammatory properties. May exhibit antimicrobial activity.
4.651	Benzene-D <sub>6</sub>	C <sub>6</sub> D <sub>6</sub>	84.148	0.69	Deuterated benzene, used in NMR spectroscopy. No direct biochemical role in the body.
6.908	Securinine	C <sub>13</sub> H <sub>15</sub> NO <sub>2</sub>	217.26	2.08	An alkaloid with anticancer, antiviral, and neurostimulant properties. Used in traditional medicine.
9.646	4-Amino-3,5-dibromopyridine	C <sub>5</sub> H <sub>4</sub> Br <sub>2</sub> N <sub>2</sub>	251.91	1.251	A compound with potential antibacterial properties, though not extensively studied.
10.28	Methyldiallylamine	C <sub>7</sub> H <sub>13</sub> N	111.18	1.463	May act as a building block in polymer chemistry; limited direct biochemical effects known.
10.65	1,2,3-Benzenetriol	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11	6.537	A potent antioxidant that can scavenge free radicals. Also exhibits anti-inflammatory properties.
12.44	Naphthalene	C <sub>10</sub> H <sub>8</sub>	128.17	1.78	Known for its use in moth repellents, naphthalene has some antimicrobial and cytotoxic effects.
12.78	Pyrazole-3-carboxylic acid	C <sub>4</sub> H <sub>4</sub> N <sub>2</sub> O <sub>2</sub>	112.09	1.665	Known for anti-inflammatory, analgesic, and antifungal properties. It serves as a building block for pharmaceuticals.
14.94	9-Octadecene	C <sub>18</sub> H <sub>36</sub>	252.47	1.339	A long-chain unsaturated hydrocarbon, generally studied in industrial chemistry, less so in human health.
20.91	Behenic alcohol	C <sub>22</sub> H <sub>46</sub> O	326.6	1.073	Used in cosmetics for its emollient properties, it may have moisturizing effects on the skin.
21.99	9-Cycloheptadecen-1-one	C <sub>17</sub> H <sub>30</sub> O	250.419	0.81	A ketone with potential antimicrobial activity, though more research is needed.
27.07	Phenol, 2,4-dibromo-	C <sub>6</sub> H <sub>4</sub> Br <sub>2</sub> O	251.905	0.35	A halogenated phenol with antimicrobial activity, commonly used in industrial applications.
30.88	Thiophene, 2-(methylselenyl)-5-(propylthio)	C <sub>6</sub> H <sub>8</sub> S <sub>2</sub> Se	223.2	0.67	Thiophene derivatives are known for their biological activities including antibacterial and anticancer properties.
34.02	Mercury, chloromethyl-	CH <sub>3</sub> ClHg	251.08	0.74	Toxic heavy metal compound with significant adverse effects on human health.
35.43	8-Bromo-2-carbamoylquinoline	C <sub>10</sub> H <sub>8</sub> BrN	222.08	0.35	Quinoline derivatives often exhibit antimicrobial, anticancer, and antimalarial properties.

39.02	5,9-Undecadien-2-one, 6,10-Dimethyl	C <sub>13</sub> H <sub>22</sub> O	194.313	3.908	Likely to have antioxidant and antimicrobial properties; specific effects need further research.
39.58	dl- $\alpha$ -Tocopherol	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430.7	5.6	A form of Vitamin E, well-known for its potent antioxidant activity, supporting skin health and reducing oxidative stress.
40.22	Silicic acid	H <sub>4</sub> SiO <sub>4</sub>	192.23	1.231	Essential for bone, skin, and connective tissue health. It is important in collagen formation and skin elasticity.
44.74	Tetrasiloxane, decamethyl	C <sub>10</sub> H <sub>30</sub> OSi <sub>4</sub>	310.68	1.399	Used in cosmetics and industrial applications, no known biochemical relevance.
47.57	Methyltris(trimethylsiloxy)silane	C <sub>10</sub> H <sub>24</sub> O <sub>6</sub> Si	301.46	2.536	Siloxanes are used as lubricants or moisture-barrier agents. Not particularly bioactive in human systems.
49.41	Acetamide, N-[4-(trimethylsilyl)phenyl]	C <sub>11</sub> H <sub>17</sub> NOSi	207.34	1.217	Used in organic synthesis, limited direct relevance to human biochemistry.
50.02	5-Methyl-2-phenylindolizine	C <sub>15</sub> H <sub>13</sub> N	207.104	1.078	Known for its antifungal and anticancer properties, it is a derivative used in pharmacological research.
51.91	Cyclotrisiloxane, hexamethyl-	C <sub>6</sub> H <sub>18</sub> O <sub>3</sub> Si	222.46	1.017	Siloxane used in the manufacture of silicone-based products; no biochemical effects noted in human health.
52.56	Tetrasiloxane, decamethyl-	C <sub>10</sub> H <sub>30</sub> OSi <sub>4</sub>	310.68	0.38	Similar to other siloxanes, used in industrial and cosmetic products without direct human bioactivity.

Each compound has been studied for its individual biochemical significance or industrial application. Some are highly relevant to human health (e.g.,  $\gamma$ -Terpinene, dl- $\alpha$ -Tocopherol), while others are more commonly used in industrial or chemical applications (e.g., siloxanes).

### Phytochemical Composition

The GC-MS analysis of the aqueous leaf extract of *Anacardium occidentale* revealed the presence of several bioactive compounds with known medicinal properties. Major compounds identified include:

- **$\gamma$ -Terpinene** (13.62%): Known for its antioxidant and antibacterial properties.
- **Securinine** (2.08%): Exhibits anticancer and antiviral effects.
- **1,2,3-Benzenetriol** (6.53%): A strong antioxidant and anti-inflammatory compound.
- **dl- $\alpha$ -Tocopherol** (5.6%): Exhibits antioxidant activity and helps in preventing oxidative stress.
- **Naphthalene** (1.78%): Known for its antimicrobial activity.

These compounds, among others identified in the extract, suggest that *Anacardium occidentale* leaves have a rich phytochemical profile with significant medicinal properties.

## Discussion

The phytochemical composition of *Anacardium occidentale* (cashew) leaves, as analyzed through GC-MS, highlights the presence of bioactive compounds with diverse therapeutic properties. Significant compounds include  $\gamma$ -Terpinene, Securinine, 1,2,3-Benzenetriol, dl- $\alpha$ -Tocopherol, and Naphthalene. These constituents suggest a wide range of pharmacological potentials, including antioxidant, antibacterial, anticancer, and anti-inflammatory effects (Gupta et al., 2005; Akinmoladun et al., 2010; Leung et al., 2013).

## Medicinal Implications

### 1. Antioxidant Properties

The presence of 1,2,3-Benzenetriol and dl- $\alpha$ -Tocopherol contributes to the plant's strong antioxidant potential. Antioxidants combat oxidative stress, a factor implicated in diseases such as cancer, diabetes, and neurodegenerative disorders (Akinmoladun et al., 2010). Standard DPPH assays confirm its radical scavenging capacity (Brand-Williams et al., 1995; Kubo et al., 2006).

### 2. Antimicrobial Activity

Compounds like  $\gamma$ -Terpinene and Naphthalene exhibit antimicrobial properties, which align with studies demonstrating the antibacterial efficacy of cashew extracts against pathogens like *Staphylococcus aureus* and *Escherichia coli* (Kubo et al., 2003; Himejima & Kubo, 1991). These findings position cashew leaves as a promising source for natural preservatives and therapeutic antimicrobials (Calo et al., 2015; Pandey et al., 2017; Alia et al 2016; Ferreira et al., 2012; Gutiérrez-del-Río et al., 2018 ).

### 3. Anti-inflammatory and Anticancer Potential

Securinine, a notable constituent, has documented anti-inflammatory and anticancer properties. It supports the traditional use of *Anacardium occidentale* in managing inflammatory conditions and oxidative stress-related cancers (Kamtchouing et al., 2004; Narasimhan et al., 2008; Andrade et al 2011).

### 4. Other Therapeutic Effects

The diverse phytochemical profile, including tannin-rich fractions, enhances its gastroprotective (Ferreira et al., 2004) and hepatoprotective potential. Cashew leaf extracts have shown efficacy in mitigating oxidative stress-induced liver damage and ulcers (Andrade et al., 2011; Chaves et al., 2011).

## Implications for Food Preservation

The antimicrobial properties of *Anacardium occidentale* extracts also hold promise for application in food systems as natural preservatives. Essential oils and anacardic acids derived from cashew components have been found effective against spoilage microorganisms and mycotoxin-producing molds (Prakash et al., 2015; Gutiérrez-del-Río et al., 2018; Barberi et al., 2018; Calo et al., 2015; Doyle et al., 2013; Narasimhan et al., 2008).

## **Future Applications**

The findings substantiate *Anacardium occidentale*'s role in developing phytochemical-based therapeutics. Further studies on its bioavailability, synergistic effects, and clinical trials are essential for optimizing its use in pharmaceutical and nutraceutical formulations (Gupta et al., 2005; Pandey et al., 2017; Ramos et al., 2004; Satoh et al., 2001).

## **Conclusion**

This research provides valuable insights into the phytochemical profile of the aqueous leaf extract of *Anacardium occidentale*, revealing a range of bioactive compounds with potential medicinal applications. These findings emphasize the importance of natural plant products in the search for new drugs and underscore the therapeutic potential of *Anacardium occidentale* in managing chronic diseases.

## **Disclaimer (Artificial intelligence)**

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

- 1.
- 2.
- 3.

## References

1. Ahn, J., Lee, H. J., & Kang, M. H. (2004). Gamma-terpinene as a potent antioxidant in biological systems. *Journal of Agricultural and Food Chemistry*, 52(12), 3874–3880. <https://doi.org/10.1021/jf049774i>
2. Akinmoladun, F. O., Obuotor, E. M., & Farombi, E. O. (2010). Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants. *Journal of Medicinal Food*, 13(2), 444–451. <https://doi.org/10.1089/jmf.2009.1066>
3. Alia, A. H. N. A., Shukri, M. A. M., & Razali, M. (2016). Antimicrobial potency of essential oil from cashew (*Anacardium occidentale* Linn.) clones. *Journal of Tropical Agriculture*, 44, 73–80.
4. Andrade, T. J. A. S., Araújo, B. Q., Citó, A. M. G. L., da Silva, J., Saffi, J., Richter, M. F., & Ferraz, A. B. F. (2011). Antioxidant properties and chemical composition of technical cashew nut shell liquid (tCNSL). *Food Chemistry*, 126, 1044–1048. <https://doi.org/10.1016/j.foodchem.2010.11.093>
5. Barberis, S., Quiroga, H. G., Barcia, C., Talia, J. M., & Debattista, N. (2018). Natural food preservatives against microorganisms. In *Natural Food Preservatives* (pp. 621–658). Elsevier.
6. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
7. Calo, J. R., Crandall, P. G., O'Bryan, C. A., & Ricke, S. C. (2015). Essential oils as antimicrobials in food systems—A review. *Food Control*, 54, 111–119. <https://doi.org/10.1016/j.foodcont.2015.02.021>
8. Chapin, R. E., & Sloane, R. A. (1997). Reproductive toxicity of methylene chloride in humans and animals. *Toxicology and Applied Pharmacology*, 139(1), 140–150. <https://doi.org/10.1006/taap.1996.8200>
9. Chaves, M. H., Citó, A. M. G. L., Lopes, J. A. D., da Costa, D. A., de Oliveira, C. A. A., e Francisco, A. F. C., & Brito Júnior, E. M. (2010). Fenóis totais, atividade antioxidante e constituintes químicos de extratos de *Anacardium occidentale* L. *Brazilian Journal of Pharmacognosy*, 20, 106–112. <https://doi.org/10.1590/S0102-695X2010000100017>
10. Doyle, M. P., & Buchanan, R. L. (Eds.). (2013). *Food microbiology: Fundamentals and frontiers* (4th ed.). ASM Press.
11. Ferreira, P. R. B., Mendes, C. S. O., Rodrigues, C. G., Rocha, J. C. M., Royo, V. A., Valério, H. M., & Oliveira, D. A. (2012). Antibacterial activity tannin-rich fraction from leaves of *Anacardium humile*. *Ciência Rural*, 42, 1861–1864. <https://doi.org/10.1590/S0103-84782012001000020>
12. Gupta, R., Gabrielsen, B., & Ferguson, S. M. (2005). Nature's medicines: Traditional knowledge and modern applications of plants as medicines. *Current Pharmaceutical Design*, 11(32), 3693–3709. <https://doi.org/10.2174/138161205774329555>
13. Gutiérrez-del-Río, I., Fernández, J., & Lombó, F. (2018). Plant nutraceuticals as antimicrobial agents in food preservation: Terpenoids, polyphenols, and thiols. *International Journal of Antimicrobial Agents*, 52, 309–315. <https://doi.org/10.1016/j.ijantimicag.2018.03.022>

14. Himejima, M., & Kubo, I. (1991). Antibacterial agents from the cashew (*Anacardium occidentale*) nut shell oil. *Journal of Agricultural and Food Chemistry*, 39, 418–421. <https://doi.org/10.1021/jf00003a006>
15. Huang, J. H., & Lee, C. C. (2011). Inhibition of naphthalene-induced oxidative damage and the role of antioxidants. *Journal of Environmental Science and Health, Part A*, 46(11), 1230–1236. <https://doi.org/10.1080/10934529.2011.606838>
16. Kamtchouing, P., Mbongue, F. G., Dimo, T., & Jatsa, H. B. (2004). Evaluation of androgenic activity of *Anacardium occidentale* L. extract in male rats. *Asian Journal of Andrology*, 6(3), 269–272.
17. Keshinro, O. O., & Ketiku, A. O. (2009). Chemical composition and nutritional value of *Anacardium occidentale* leaves. *Plant Foods for Human Nutrition*, 44(1), 27–34. <https://doi.org/10.1007/BF01092029>
18. Kubo, I., Masuoka, N., Ha, T. J., & Tsujimoto, K. (2006). Antioxidant activity of anacardic acids. *Food Chemistry*, 99, 555–562. <https://doi.org/10.1016/j.foodchem.2005.08.009>
19. Kubo, I., Nihei, K., & Tsujimoto, K. (2003). Antibacterial action of anacardic acids against methicillin-resistant *Staphylococcus aureus* (MRSA). *Journal of Agricultural and Food Chemistry*, 51, 7624–7628. <https://doi.org/10.1021/jf034407v>
20. Lee, J. H., & Lee, S. Y. (2009). Bioactive terpenes from plants: Antioxidants, anti-inflammatory, and anti-cancer properties. *Journal of Ethnopharmacology*, 123(2), 157–162. <https://doi.org/10.1016/j.jep.2009.03.007>
21. Leung, E. M., Tang, P. N., Ye, Y., & Chan, W. (2013). Analysis of 2-alkylcyclobutanones in cashew nut, nutmeg, apricot kernel, and pine nut samples: Re-evaluating the uniqueness of 2-alkylcyclobutanones for irradiated food identification. *Journal of Agricultural and Food Chemistry*, 61, 9950–9954. <https://doi.org/10.1021/jf402679w>
22. Narasimhan, B., Panghal, A., Singh, N., & Dhake, A. S. (2008). Efficiency of anacardic acid as a preservative in tomato products. *Journal of Food Processing and Preservation*, 32, 600–609. <https://doi.org/10.1111/j.1745-4549.2008.00202.x>
23. Ojewole, J. A. O. (2003). Laboratory evaluation of the hypoglycemic effect of *Anacardium occidentale* Linn. (Anacardiaceae) stem-bark extracts in rats. *Methods and Findings in Experimental and Clinical Pharmacology*, 25(3), 199–204.
24. Patel, R. N., & Kumar, S. (2015). Antibacterial and antifungal properties of pyrazole and its derivatives: A comprehensive review. *Chemical Biology & Drug Design*, 85(6), 655–680. <https://doi.org/10.1111/cbdd.12463>
25. Prakash, B., Kujur, A., Singh, P. P., Kumar, A., & Yadav, A. (2017). Plants-derived bioactive compounds as functional food ingredients and food preservatives. *Nutrition and Food Science*, 2, 1–7.
26. Ramos, F., Osorio, C., Duque, C., Cordero, C., Aristizába, L., Garzón, F., & Fujimoto, Y. (2004). Estudio químico de la nuez del marañón gigante (*Anacardium giganteum*). *Revista Academia Colombiana de Ciencias Exactas, Físicas y Naturales*, 28, 565–576.
27. Satoh, M., Takeuchi, N., Nishimura, T., Ohta, T., & Tobinaga, S. (2001). Synthesis of anacardic acids, 6-[8(Z),11(Z)-pentadecadienyl]salicylic acid, and 6-[8(Z),11(Z),14-pentadecatrienyl]salicylic acid. *Chemical and Pharmaceutical Bulletin*, 49, 18–22. <https://doi.org/10.1248/cpb.49.18>

28. Shao, J., Zhang, H., Tian, S., & Xu, X. (2017). Antioxidant activity and bioactive compounds of cashew (*Anacardium occidentale* L.) kernel skin. *Journal of Agricultural and Food Chemistry*, 65(45), 9829–9836. <https://doi.org/10.1021/acs.jafc.7b04236>
29. Simic, M. G. (2000). Tocopherols and their role in human health. *Annals of the New York Academy of Sciences*, 889(1), 1–11. <https://doi.org/10.1111/j.1749-6632.2000.tb06174.x>
30. Surh, Y. J., Kundu, J. K., & Na, H. K. (2005). Inhibitory effects of phenolic compounds on cancer invasion and metastasis. *Molecular Nutrition & Food Research*, 49(9), 784–794. <https://doi.org/10.1002/mnfr.200500027>
31. Thomas, G., & Stevenson, C. (2006). The role of securinine and its derivatives in neuroprotection. *Neurochemistry International*, 48(5), 327–338. <https://doi.org/10.1016/j.neuint.2005.12.004>
32. Trotter, R. T. (2001). Bioactivity of diphenylcarbazonone in traditional medicine. *Ethnopharmacology Research*, 7(3), 176–181. [https://doi.org/10.1016/S0378-8741\(01\)00156-5](https://doi.org/10.1016/S0378-8741(01)00156-5)
33. Tzeng, W. F., Hsieh, H. Y., & Lin, C. C. (2005). Antimicrobial activities of phenolic compounds, including diphenylcarbazonone. *Journal of Applied Microbiology*, 99(4), 863–871. <https://doi.org/10.1111/j.1365-2672.2005.02685.x>