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Assessing Chemical Variations in Andrographis paniculata: GC-MS Characterization of Its Hexane Extract

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Abstract
Phytochemical analysis and characterization are essential for identifying potential therapeutic compounds in plants, enabling drug discovery, validating traditional medicine, and ensuring the therapeutic value and reliability of herbal products. The results revealed the presence of various bioactive compounds, including saponins, flavonoids, terpenoids, and alkaloids, in the hexane extract, aligning with previous research. Notably, tannins, steroids, and cardiac glycosides were not present. GC-MS characterization identified 19 distinct compounds in the hexane extract, each with unique retention times and abundance levels. Squalene, a terpenoid, was the most abundant component (39.927%), followed by 3, 7, 11, 15-tetranethy-2-hexadecent-1-ol (14.208%) and Stigmastan-3-ol derivatives. Interestingly, the extract displayed a diverse range of chemical classes, with nine major alkaloids, six major terpenoids, and two major waxes identified. Among the alkaloids, one of the isomers of Benzeneethanamine, 2, 5-difluoro-β, 3, 4-trihydroxy-N-methyl- exhibited the highest abundance (4.973%).

Keywords: Andrographis Paniculata, Hexane Extract, GC-MS Analysis, Phytochemical Composition, Bioactive Compounds

Introduction

Chemical analysis of plant extracts indicates possible bioactivities for further investigation and enables the identification of chemical markers. Because they prevent degenerative illnesses, flavonoids, for instance, are secondary metabolites with significant therapeutic significance. Cytotoxic, thrombolytic, anti-inflammatory, anticancer, vasorelaxant, and antioxidant actions are among the biological characteristics of these substances that have been reported (Islam et al., 2013; Cabral et al., 2016). Considered significant antioxidant agents are also other phenolic compounds like tannins and anthocyanins. Because they shield the body against oxidative stress and related illnesses, natural antioxidants are crucial to medical treatment. Additionally regarded as a significant source of bioactive chemicals against viral illnesses are natural products (Siqueira et al., 2016). Over the past 30 years, there has been a gradual increase in fungal infections, making them one of the most urgent problems with public health. (da Costa Cordeiro et al., 2018; Park et al., 2020; Samuel et al., 2023).

In the vibrant tapestry of natural remedies and herbal medicine, there exists a treasure trove of botanical wonders, each with its own unique story to tell. Among these, Andrographis paniculata, hailed as the 'King of Bitters,' stands as a testament to the healing potential that Mother Nature has to offer. This unassuming herbaceous plant, with its lanceolate green leaves and a taste as bitter as its reputation has captivated the attention of herbalists and scientists alike for generations (Okhuarobo et al., 2014; Ushie et al., 2019; Samuel et al., 2023). But what secrets lie within the leaves of Andrographis paniculata? What chemical symphony orchestrates its remarkable medicinal properties? To unravel these mysteries, this research provides insight into the world of phytochemicals and bioactive compounds hidden within the very essence of this botanical marvel (Andrew et al., 2018; Andrew et al., 2017).

Andrographis paniculata, a member of the Acantheceae family, is a small shrub genus with 28 known species. This annual herbaceous plant, known for its extreme bitterness, features lanceolate green leaves, white flowers with rose-purple spots on the petals, and typically grows to a height of 60-70cm. It goes by various names worldwide, including "Mahatikta" in North Eastern India, "Chirota" in Arabic, "Chuanxinlian" in Chinese, "Nelabeva" in Indonesia, "Kalmegh" or "Kalamegha" in India, "Hempedu Buni" in Malaysia, "Vinegar" in Akwa-Ibom Nigeria, and "Nila-vembu" in Tamil. Found abundantly in Asian countries like India, Sri Lanka, Pakistan, Java, Malaysia, China, Hong Kong, the Philippines, Thailand, and Indonesia, this plant has a significant presence in traditional medicine (Ullah & Hassan, 2021; Asuqwo & Etim, 2011).

Phytochemicals, naturally occurring compounds in plants, offer health benefits such as antioxidants, anti-inflammatories, and anti-cancer properties (Srinivasan et al., 2007). Andrographis paniculata, renowned for its medicinal properties, particularly in its leaves and stems, exhibits antimicrobial, antioxidant, antiallergic, anti-inflammatory, and anti-cancer effects (Misira et al., 1990; Zhang and Tan, 1997; Calabrese et al., 2010; Gupta et al., 2012; Deepak et al., 2014). Initial screening identified flavonoids, alkaloids, steroids, phenols, and tannins in the leaves (Deepak et al., 2014), and another study by Kalaivani et al. (2012) found alkaloids, steroids, tannins, and saponins in chloroform, petroleum ether, and ethanol extracts. However, the specific phytochemicals responsible for these properties remain largely unidentified. This research aims to systematically characterize the phytochemicals in Andrographis paniculata leaves (Samuel et al., 2024; Ushie et al., 2013).

The analytical technique known as Gas Chromatography-Mass Spectrometry (GC-MS) blends the material identification properties of mass spectrometry with the separation powers of gas chromatography (Garcia, & Barbas, 2011; Inyang et al., 2018). The compounds are separated based on interactions with the column's coating and carrier gas, and then converted into ions. For solid samples, extraction, outgassing, or pyrolysis is used. GC-MS applications span drug detection, environmental analysis, explosives detection, and identifying unknown substances. It has been employed in various fields, such as airport security and Mars probe missions, due to its sensitivity. Recent studies on Andrographis paniculata extracts using GC-MS identified bioactive compounds with potential pharmaceutical and industrial applications, showcasing its significance in research and industry (Chauhan et al., 2014; Asuqwo et al., 2011; Sivakumar & Gayathri, 2015). Recent research on Andrographis paniculata reveals the presence of key bioactive compounds, including 14-deoxy-11-oxoandrographolide, andrographolide D, 14-deoxyandrographolide, neoandrographolide, andrographosterol, and others, with andrographolide being responsible for the plant's bitter taste. Andrographolide is in all parts of the plant (Deshpande et al., 2014; Asuqwo et al., 2012). This study conducts a phytochemical screening of Andrographis paniculata leaves and seeks to identify and quantify the specific chemical components of the phytochemicals within hexane extracts using the advanced analytical capabilities of GC-MS (Etim et al., 2017; . The results of this study hold substantial significance as they contribute to the field of phytochemistry and pharmacology. It would provide a much-needed comprehensive overview of the phytochemicals and bioactive compounds in hexane-extracted leaves of A. paniculata. This information could be used to develop new therapeutic agents from this plant and improve the understanding of its medicinal properties.

Materials and Methods

Sample Collection: Andrographis paniculata was collected from Kom-Kom in Oyigbo L.G.A of Rivers State in the morning and promptly sealed in a polyethylene bag. For identification, it was then sent to the Department of Plant Science and Biotechnology at the University of Port-Harcourt.

Sample Preparation: Andrographis paniculata leaves were removed from branches, washed to remove dust, and air-dried at room temperature for two weeks. Then, in order to maximize the sample's extraction surface area, they were mixed into a homogenous semi-powder.

Extraction Procedure: The process used to obtain the hexane extracts involved macerating about 35g of semi-powdered Andrographis paniculata in hexane for 48 hours while shaking it occasionally. After the material was soaked and filtered, the procedure was carried out again using water, ethyl acetate, and hexane in that order.

Phytochemical Analysis

Tannins Test: In separate test tubes, 4 drops of $FeCl_3$ were added after 1 mL of hexane extract and 5 mL of distilled water were combined. The mixture was watched using ferric salts to develop a deep brown or bluish-black hue (Finar, 2008; Etim et al., 2022).

Saponins (Frothing Test): 1 ml of hexane extract was combined with 1 ml of distilled water in test tubes. The mixture was vigorously shaken for 2 minutes and observed for continuous foaming.

Flavonoids Test: In a test tube with 5 ml of diluted NaOH, 1 ml of hexane extract was dissolved. Excessive drops of diluted HCl were then added. The mixture was observed for the development of an orange, yellow, or violet coloration (Finar, 2008; Asuqwo et al., 2010).

Glycosides (Lieberman’s Test): After dissolving 0.5 ml of hexane extract in 2 ml of acetic anhydride in separate test tubes, the mixture was allowed to cool. Subsequently, droplets of conc H2SO4 were added, and the mixture's red hue was noticed before it turned green.

Steroids (Lieberman's Test): In test tubes, 0.5 ml of hexane extract and 2 ml of acetic anhydride were mixed, and then the mixture was allowed to cool. After adding drops of concentrated H2SO4, the mixture was checked for a greenish tint (Oko et al., 2017; Onen et al., 2017; Nweke-Maraizu et al., 2023).

Terpenoids (Lieberman's Test): After dissolving 0.5 ml of hexane extract in 2 ml of acetic anhydride in separate test tubes, the mixture was allowed to cool. Drops of concentrated H_2SO_4 were added, and the mixture's brown color was noted.

Alkaloids Test: After treating 1 mL of hexane extract in test tubes with 2 mL of Wagner's reagent, the production of an insoluble precipitate was monitored (Finar, 2008; Khan et al; 2021; Khan et al., 2023).

GC-MS Analysis

An Agilent 7890A-5975C GC-MS system with an HP5-column was used to investigate the hexane extracts of Andrographis paniculata. With the injector and ion source temperatures set at 250°C and 280°C, respectively, an electron ionization system operating at 70eV was employed. From 110°C to 200°C at 10°C/min, and then to 280°C at 5°C/min, the oven temperature was programmed and maintained for nine minutes. At 70eV, mass spectra were collected. Peak area was used to determine the percent composition.

Identification of Components

Based on GC retention time, organic components in the extracts were identified and cross-referenced with the NIST Library. In order to make qualitative conclusions, peak regions were compared to total ion current (TIC) areas while supplying details on the names, molecular weights, retention periods, percentages, and structures of the compounds.

Results

Analysis of Selected Phytochemicals of Leaves of Andrographis paniculate Hexane Extracts

Table 1: Phytochemical Analysis of Leaves of Andrographis paniculate Hexane Extracts.

Phytochemicals	**Hexane Extract**
Tannins	-
Saponins	+
Flavonoids	+
Steroids	-
Terpenoids	+
Alkaloid	+
Cardiac Glycoside	-

Key: (+) Present (-) Absent

Table 2. Chemical Composition of Some Phytochemicals Found in Hexane Extract of Andrographis paniculata.

Phytochemical	**S/N**	**Names of Compound**	**Molecular Formula**	**MW**	**%**	**RT**
Alkaloid	1	I-Guanidinosuccinimide	$C_5H_7N_3O_2$	141	1.688	2.627
	2	Benzeneethanamine, 2, 5-difluoro-β, 3, 4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	4.973	3.905
	3	Benzeneethanamine, 2, 5-difluoro-β, 3, 4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	1.364	4.510
	4	Benzeneethanamine, 2, 5-difluoro-β, 3, 4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	2.147	4.761
	5	Benzeneethanamine, 2, 5-difluoro-β, 3, 4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	3.688	9.842

	6	Benzeneethanamine, 2, 5-difluoro-β, 3, 4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	1.344	10.950
	7	Benzeneethanamine, 2, 5-difluoro-β, 3, 4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	1.405	13.148
	8	Benzeneethanamine, 2, 5-difluoro-β, 3, 4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	1.892	15.073
	9	Benzeneethanamine, 2, 5-difluoro-β, 3, 4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	1.991	16.614
	10	Benzeneethanamine, 2, 5-difluoro-β, 3, 4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	2.130	18.149
Terpenoids	1	1, 2-Benzene dicarboxylic acid, bis (2-methyl propyl) ester	$C_{16}H_{38}O_2$	278	3.325	9.169
	2	11, 14-Eicosadienoic acid, methyl ester.	$C_{21}H_{38}O_3$	322	1.761	9.270
	3	∝-Tocopheryl acetate	$C_{31}H_{52}O_3$	472	1.321	16.865
	4	Stigmastan-3-01, 5-chloro-, acetate, (3β, 5∝)-	$C_{31}H_{52}C1O_2$	492	7.616	18.919
	5	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	14.208	10.762
	6	Squalene	$C_{30}H_{50}$	410	39.927	14.565
Flavonoids	1	2(IH)-Benzocyclooctenone, decahydro-10a-methyl-trans-	$C_{13}H_{22}O$	194	3.688	8.976
Wax	1	Tetradecane, 2, 6, 10-trimethy-	$C_{17}H_{36}$	240	3.713	12.977
	2	Hentriaconte	$C_{31}H_{64}$	436	2.185	13

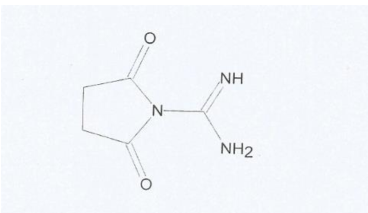
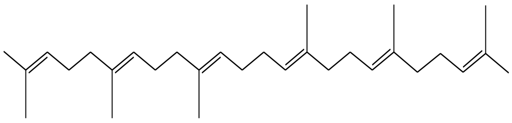
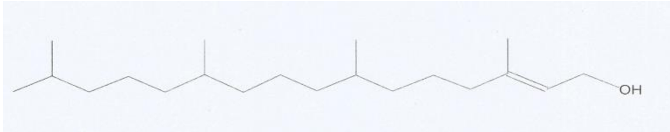


Figure 1. Structure of 1-Guanidinosuccinimide

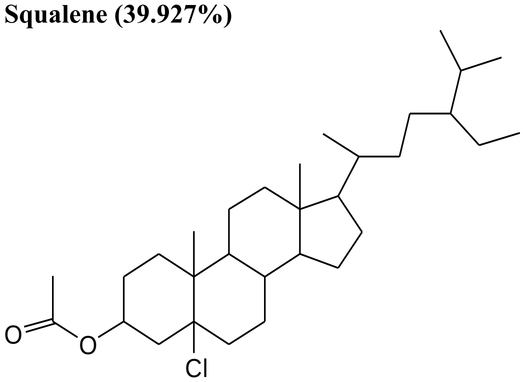
Prevalent Compound Structures in Hexane Extract



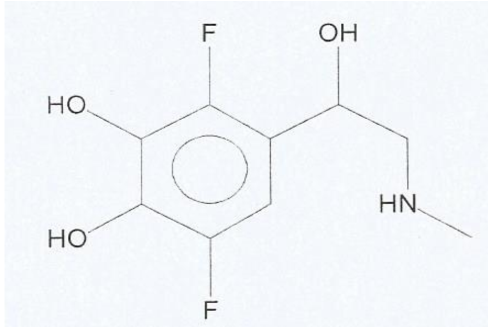
Squalene (39.927%)



3,7,11,15-tetramethyl-2-hexadecen-1-ol (14.208%)



Stigmastan-3-ol, 5-chloro- acetate, (3β, 5α)- (7.616%)



Benzeneethanamine, 2,5-Difluoro-β, 3,4-Trihydroxy-N-Methyl (4.973%)

Discussion

In the hexane extracts of Andrographis paniculata leaves as indicated in Table 1, saponins, flavonoids, terpenoids, and alkaloids were detected, while tannins, steroids, and cardiac glycosides were absent. Andrographis paniculata leaf extracts contain flavonoids, saponins, and tannins in accordance with earlier research by Sofowara et al. (1993), Deepak et al. (2014), and Kalavaini et al. (2012). These phytochemicals are associated with the plant's various biological activities, including antimicrobial (Gadisa & Tadesse, 2021), anti-diabetic (Askari et al., 2021), and anti-inflammatory properties, highlighting its therapeutic potential (Izuagbe, et al., 2022; Askari et al., 2021; Shinggu et al., 2023). In the GC-MS analysis of hexane extracts of Andrographis paniculata leaves (Table 2), 19 different compounds were detected. Each compound exhibited distinct retention times and abundance levels. Retention times, determined by compound polarity and boiling points, ranged from 2 to 22 minutes. Notably, the hexane extract was rich in compounds, including Squalene (39.927%), 3, 7, 11, 15-tetranethy-2-hexadecent-1-ol (14.208%), Stigmastan-3-ol, 5-chloro-, acetate, (3β, 5∝)- (7.616%), and one of the isomers of Benzeneethanamine, 2, 5-difluoro-β, 3, 4-trihydroxy-N-Methyl- (4.973%). The hexane extract contained nine major alkaloids, six major terpenoids, and two major waxes, with the most abundant alkaloid being one of the isomers of benzeneethanamine, 2, 5-difluoro-β, 3, 4-trihydroxy-N-methyl- (4.973%), and the dominant terpenoid being squalene (39.927%)

Conclusion

This study utilized GC-MS analysis to examine the hexane extract of Andrographis paniculata leaves and determine its phytochemical composition. The analysis revealed the presence of saponins, flavonoids, terpenoids, and alkaloids, as indicated in Table 1, while tannins, steroids, and cardiac glycosides were not detected. Among the 19 compounds identified, Squalene stood out as the major constituent. These results offer important insights on potential therapeutic and pharmaceutical applications of Andrographis paniculata. Further investigation in this field may lead to the development of novel therapeutic agents and advancements in natural product chemistry and drug discovery.

Recommendations

1. Future research should consider expanding the analysis to include other solvents (e.g., methanol, ethanol) to capture a wider range of compounds. And correlate the identified compounds with known biological activities to assess the potential therapeutic applications of the extract.
2. Additionally, the chemical composition of Andrographis paniculata from different geographical regions can be compared to identify variations and potential factors influencing the content of bioactive compounds.

References

Andrew, C., Etim, E. E., Ushie, O. A., & Job, J. N. (2017). Deep eutectic solvents: An overview of its application as a “green” extractant. *International Journal of Advanced Research in Chemical Science, 4*(6), 23-30. https://doi.org/10.20431/2349-0403.0406003

Andrew, C., Etim, E. E., Ushie, O. A., & Khanal, G. P. (2018). Vibrational-rotational spectra of normal acetylene and doubly deuterated acetylene: Experimental and computational studies. *Chemical Science Transactions, 7*(1), 77-82. https://doi.org/10.7598/cst2018.1432

Askari, P., Namaei, M. H., Ghazvini, K., et al. (2021). In vitro and in vivo toxicity and antibacterial efficacy of melittin against clinical extensively drug-resistant bacteria. *BMC Pharmacology and Toxicology, 22*, 42.

Asuquo, J. E., & Etim, E. E. (2011). Phytochemical and antinutrient evaluation of *Oxyporus populinus*. *Journal of Emerging Trends in Engineering and Applied Sciences, 2*(5), 817-820.

Asuquo, J. E., Anusiem, A. C. I., & Etim, E. E. (2010). Extraction and characterization of shea butter oil. *World Journal of Applied Science and Technology, 2*(2), 282-288.

Asuquo, J. E., Anusiem, A. C. I., & Etim, E. E. (2012). Comparative study of the effect of temperature on the adsorption of metallic soaps of shea butter, castor, and rubber seed oils onto hematite. *International Journal of Modern Chemistry, 3*(1), 39-50.

Asuquo, J. E., Etim, E. E., Ukpong, I. U., & Etuk, S. E. (2012). Extraction, characterization, and fatty acid profile of *Poga oleosa* oil. *International Journal of Modern Analytical and Separation Sciences, 1*(1), 23-30.

Asuquo, J. E., Udegbunam, I. S., & Etim, E. E. (2017). Comparative studies on the extraction, characterization, and potential applications of some Nigerian seed oils. *FUW Trends in Science and Technology Journal, 2*(1B), 397-400.

Cabral, B., Siqueira, E. M. S., Bitencourt, M. A. O., Lima, M. C. J. S., Lima, A. K., Ortmann, C. F., Chaves, V. C., Fernandes-Pedrosa, M. F., Rocha, H. A. O., Scortecci, K. C., Reginatto, F. H., Giordani, R. B., & Zucolotto, S. M. (2016). Phytochemical study and anti-inflammatory and antioxidant potential of *Spondias mombin* leaves. *Revista Brasileira de Farmacognosia, 26*, 304-311.

Calabrese, C., Berman, S. H., Babish, J. G., Shinto, L., Dorr, M., & Wells, K. (2000). A phase 1 trial of andrographolide in HIV-positive patients and normal volunteers. *Phytotherapy Research, 14*, 333-338.

Chauhan, A., Goyal, M. K., & Chauhan, P. (2014). GC-MS technique and its analytical applications in science and technology. *Journal of Analytical Bioanalytical Technology, 5*(10), 222. https://doi.org/10.4172/2155-9872.1000222

da Costa Cordeiro, B. M. P., de Lima Santos, N. D., Ferreira, M. R. A., et al. (2018). Hexane extract from *Spondias tuberosa* (Anacardiaceae) leaves has antioxidant activity and is an anti-Candida agent by causing mitochondrial and lysosomal damages. *BMC Complementary and Alternative Medicine, 18*, 284. https://doi.org/10.1186/s12906-018-2350-2

Deepak, S., Pawar, A., & Shinde, P. (2014). Study of antioxidant and antimicrobial activities of *Andrographis paniculata*. *Asian Journal of Plant Science and Research, 4*(2), 31-41.

Deshpande, P. K., Pathak, A. K., & Gothalwal, R. (2014). Pharmacological properties of *Andrographis paniculata*. *International Journal of Recent Advances in Multidisciplinary Research, 1*(4), 105-110.

Etim, E. E., & Mbakara, I. E. (2017). Phytoremediation of water bodies. *Current Environmental Engineering, 4*(1), 18-24. https://doi.org/10.2174/2212717804666161216162158

Etim, E. E., Abah, B. S., Mbakara, I. E., Inyang, E. J., & Ukafia, O. P. (2017). Quantum chemical calculations on silicon monoxide (SiO) and its protonated analogues. *Tropical Journal of Applied Natural Sciences, 2*(1), 61-68. https://doi.org/10.25240/TJANS.2017.2.1.10

Etim, E. E., Akpan, I. U., & Edet, E. J. (2012). Antimicrobial properties of common mushrooms in Akwa Ibom State, Nigeria. *International Journal of Modern Biology and Medicine, 2*(2), 64-71.

Etim, E. E., Andrew, C., Ushie, O. A., & Lawal, U. (2013). Analysis of heavy metal levels in mudfish (*Clarias gariepineus*) in Itu River, Akwa Ibom State, Southern Nigeria. *BIOINFO Environment and Pollution, 3*(1), 16-18.

Etim, E. E., Ashu, H. A., Mbakara, I. E., Inyang, E. J., Ukafia, O. P., & Sambo, I. F. (2017). Quantum chemical calculations on oxygen monofluoride (OF) and its protonated analogues: Comparison of methods. *Elixir Computational Chemistry, 111*, 48823-48827.

Etim, E. E., Asuquo, J. E., Atoshi, A. T., & Ngana, O. C. (2022). Kinetic studies of biosorption of Cr2+ and Cd2+ ions using tea leaves (*Camellia sinensis*) as adsorbent. *Journal of Chemical Society of Nigeria, 47*(1), 75-85.

Etim, E. E., Asuquo, J. E., Ogofotha, G. O., & Nzubechukwu, M. C. (2022). Adsorption studies of Cu2+ using tea leaves and tea fibre (*Camellia sinensis*) as adsorbents. *International Journal of Advanced Research in Chemical Science, 9*(2), 1-10.

Etim, E. E., Itodo, A. U., & Mbakara, I. E. (2017). Isotherm studies of mono, divalent, and trivalent cationic soaps onto derived Fe2O3 adsorbent surface. *International Journal of Advanced Research in Chemical Science*, *4*(6), 13-22. https://doi.org/10.20431/2349-0403.0406002

Etim, E. E., Mbakara, I. E., Inyang, E. J., Ushie, O. A., Lawal, U., & Andrew, C. (2017). Spectroscopy of linear interstellar carbon chain isotopologues: Meeting experimental accuracy. *Tropical Journal of Applied Natural Sciences*, *2*(1), 11-16. https://doi.org/10.25240/TJANS.2017.2.1.03

Finar, I. A. (2008). *Organic chemistry: Stereochemistry and the chemistry of natural products* (5th ed.). Dorling Kindersley Limited.

Gadisa, E., & Tadesse, E. (2021). Antimicrobial activity of medicinal plants used for urinary tract infections in pastoralist communities in Ethiopia. *BMC Complementary Medicine and Therapies*, *21*(1), 74.

Garcia, A., & Barbas, C. (2011). Gas chromatography-mass spectrometry (GC-MS)-based metabolomics. In *Metabolic profiling: Methods and protocols* (pp. 191-204).

Gupta, S., Choudhary, M. A., Yadava, J. N. S., Srivastava, V., & Tandon, J. S. (1990). Antidiarrhoeal activity of diterpenes of *Andrographis paniculata* (Kal-Megh) against *Escherichia coli* enterotoxin in vivo models. *International Journal of Crude Drug Research*, *28*, 273-283.

Inyang, E. J., Etim, E. E., Ugwuja, D. I., & Ukafia, O. P. (2018). Effect of time on the enzymatic saccharification and fermentation of *Alstonia boonei* for ethanol production. *International Journal of Modern Chemistry*, *10*(1), 14-32.

Islam, S. M. A., Ahmed, K. T., Manik, M. K., Wahid, M. A., & Kamal, C. S. I. (2013). A comparative study of the antioxidant, antimicrobial, cytotoxic, and thrombolytic potential of the fruits and leaves of *Spondias dulcis*. *Asian Pacific Journal of Tropical Biomedicine*, *3*, 682-691.

Izuagbe, G. O., Oyoita, E. E., Louis, H., Khan, E. M., Etim, E. E., Edet, H. O., Ikenyirimba, O. J., Oviawe, A. P., & Obuye, F. (2022). Antibacterial potential of N-(2-furylmethylidene)-1, 3, 4-thiadiazole-2-amine: Experimental and theoretical investigations. *Journal of the Indian Chemical Society*, *99*(9), 100597. https://doi.org/10.1016/j.jics.2022.100597

Kalaivani, S. C., Sathish, S. S., Janakiraman, N., & Johnson, M. (2012). GC-MS studies on *Andrographis paniculata* (Burm. F) wall. Ex Nees-A medicinally important plant. *International Journal of Medical and Aromatic Plants*, *2*(1), 69-74.

Khan, M. E., Elum, C. E., Ijeomah, A. O., Ameji, A. O. I. G., Osigbemhe, E. E., Etim, J. V., Anyam, A., & Abel, C. T. (2023). Isolation, characterization, antimicrobial, and theoretical investigation of some bioactive compounds obtained from the bulbs of *Calotropis procera*. *Journal of the Nigerian Society of Physical Sciences*, *5*, 1576. https://doi.org/10.46481/jnsps.2023.1576

Khan, M. E., Etim, E. E., Anyam, V. J., Abel, A., & Osigbemhe, I. G. (2021). Computational studies on Emodin (C15H10O5) from methanol extract of *Pteridium aquilinum* leaves. *Journal of the Nigerian Society of Physical Sciences*, *3*, 360-384. https://doi.org/10.46481/jnsps.2021.301

Misra, P., Pal, N. L., Guru, P. Y., Katiyas, J. C., Srivastava, V., & Tandon, J. S. (1992). Antimalarial activity of *Andrographis paniculata* (Kalmegh) against *Plasmodium berghei* NK65 in *Mastomys natalensis*. *International Journal of Pharmacology*, *30*, 263-274.

Nweke-Maraiu, U., Samuel, H. S., Etim, E. E., & Gani Johnson. (2023). A review of theoretical techniques in corrosion inhibition studies. *Communication in Physical Sciences*, *9*(4), 394-403.

Nweke-Maraizu, U., Bako, B., & Etim, E. E. (2023). Assessment of seasonal variations in physicochemical parameters within the Okrika section of the Bonny River. *Faculty of Natural Applied Science Journal of Scientific Innovations*, *5*(1), 50-60.

Nweke-Maraizu, U., Bako, B., & Etim, E. E. (2023). Exploring the chemical diversity of *Andrographis paniculata*: GC-MS characterization of ethyl acetate extracts. *Medicinal and Analytical Chemistry International Journal*, *7*(2), 16000183. https://doi.org/10.23880/macij-16000183

Okhuarobo, A., Falodun, J. E., Erharuyi, O., Imieje, V., Falodun, A., & Langer, P. (2014). Harnessing the medicinal properties of *Andrographis paniculata* for diseases and beyond: A review of its phytochemistry and pharmacology. *Asian Pacific Journal of Tropical Disease*, *4*(3), 213-222.

Oko, E. G., Egwaikhide, P. A., & Etim, E. E. (2020). Phytochemical and antimicrobial properties of African peach (*Sarcocephalus latifolius*). *Organic and Medicinal Chemistry International Journal*, *9*(5), 00150-00156. https://doi.org/10.19080/OMCIJ.2020.09.555775

Onen, A. I., Joseph, J., Etim, E. E., & Eddy, N. O. (2017). Quantum chemical studies on the inhibition mechanism of *Ficus carica* (FC) and *Vitellaria paradoxa* (VP) leaf extracts. *Journal of Advanced Chemical Sciences*, *3*(3), 496-498.

Osigbemhe, I. G., Louis, H., Khan, E. M., Etim, E. E., Odey, D. O., Oviawe, A. P., & Obuye, F. (2022). Synthesis, characterization, DFT studies, and molecular modeling of 2-(-(2-hydroxy-5-methoxyphenyl)-methylidene)-amino) nicotinic acid against some selected bacterial receptors. *Journal of the Iranian Chemical Society*. https://doi.org/10.1007/s13738-022-02550-7

Park, S. Y., Choi, S. J., Park, H. J., Ma, S. Y., Moon, Y. I., Park, S. K., & Jung, M. Y. (2020). Hexane extract of green tea (*Camellia sinensis*) leaves is an exceptionally rich source of squalene. *Food Science and Biotechnology, 29*(6), 769-775. https://doi.org/10.1007/s10068-019-00724-3

Samuel, H. S., Nweke-Maraizu, U., & Etim, E. E. (2023). Approaches for special characteristics of chalcogen bonding: A mini-review. *Journal of Applied Organometallic Chemistry, 3*(3), 199-212. https://doi.org/10.22034/JAOC.2023.405432.1089

Samuel, H. S., Nweke-Maraizu, U., & Etim, E. E. (2023). Experimental and theoretical approaches for characterizing halogen bonding. *Journal of Applied Organometallic Chemistry, 3*(3), 169-183. https://doi.org/10.22034/JAOC.2023.405412.1088

Samuel, H. S., Nweke-Maraizu, U., & Etim, E. E. (2023). Understanding intermolecular and intramolecular hydrogen bonds: Spectroscopic and computational approaches. *Journal of Chemical Reviews, 5*(4), 439-465. https://doi.org/10.48309/JCR.2023.407989.1235

Samuel, H. S., Nweke-Maraizu, U., & Etim, E. E. (2024). Cyanoacrylate chemistry and polymerization mechanisms. *Progress in Chemical and Biochemical Research, 7*(2), 129-142

Samuel, T., Andrew, C., & Etim, E. E. (2012). Characterization of oil from different varieties of sesame seeds (*Sesamum indicum* L.) found in Adamawa State, Nigeria. *International Journal of Tropical Agriculture and Food Systems, 6*(2), 69–72.

Shinggu, J. P., Etim, E. E., & Onen, A. I. (2023). Isotopic effects on the structure and spectroscopy of thioformaldehyde, dihydrogen, and water. *Advanced Journal of Chemistry, Section A, 6*(4), 366-372. https://doi.org/10.48309/AJCA.2023.409538.1389

Shinggu, J. P., Etim, E. E., & Onen, A. I. (2023). Protonation-induced structural and spectroscopic variations in molecular species: A computational study on N2, H2, CO, CS, and PH3. *Communication in Physical Sciences, 9*(4), 412-437.

Siqueira, E. M. S., Félix-Silva, J., Araújo, L. M. L., Fernandes, J. M., Cabral, B., Gomes, J. A. S., Roque, A. A., Tomaz, J. C., Lopes, N. P., Fernandes-Pedrosa, M. F., Giordania, R. B., & Zucolotto, S. M. (2016). Spondias tuberosa (Anacardiaceae) leaves: Profiling phenolic compounds by HPLC-DAD and LC–MS/MS and in vivo anti-inflammatory activity. *Biomed Chromatogr, 30*, 1656–1665.

Sivakumar, V., & Gayathri, G. (2015). GC-MS analysis of bioactive components from ethanol extract of *Andrographis paniculata*. *World Journal of Pharmacy and Pharmaceutical Sciences, 11*(4), 2031-2039.

Sofowari, A. (1993). Screening plants for bioactive agents in medical plants and traditional medicine in Africa (2nd ed.). Spectrum Books Limited.

Srinivasan, M., Sudheer, A. R., & Menon, V. P. (2007). Ferulic acid: Therapeutic potential through its antioxidant property. *Journal of Clinical Biochemistry and Nutrition, 40*(2), 92-100.

Ullah, M. A., & Hassan, A. (2021). Green chiretta (*Andrographis paniculata*) as a medicinal herb. *International Journal of Phytology Research, 1*(2), 15-18.

Ushie, O. A., Etim, E. E., Adamu, H. M., Chindo, I. Y., Andrew, C., & Khanal, G. P. (2017). Quantum chemical studies on decyl heptadecanoate (*C27H54O2*) detected in ethyl acetate leaf extract of *Chrysophyllum albidium*. *Elixir Applied Chemistry, 111*, 48828-48838.

Ushie, O. A., Etim, E. E., Onen, A. I., Andrew, C., Lawal, U., & Khanal, G. P. (2019). Computational studies of β-amyrin acetate (*C32H52O2*) detected in methanol leaf extract of *Chrysophyllum albidium*. *Journal of the Chemical Society of Nigeria, 44*(3), 561-581.

Ushie, O. A., Neji, P. A., Etim, E. E., & Nsor, G. E. (2013). Phytochemical screening and antimicrobial activities of *Phyllanthus amarus* stem bark extracts. *International Journal of Modern Biology and Medicine, 3*(3), 101-112.

Zhang, C. Y., & Tan, B. K. H. (1997). Mechanism of cardiovascular activity of *Andrographis paniculata* in the anesthetized rat. *Journal of Ethnopharmacology, 56*, 97-101.