# PHYTOCHEMICAL AND CYTOTOXIC EVALUATION OF THE FRUITS OF *RAUWOLFIA VOMITORIA* AFZEL. (APOCYNACEAE)


## ABSTRACT

*Rauwolfia vomitoria* fruits has been claimed tobe used traditionally in treating cancer related diseases. However, this activity has not yet been scientifically proven. This study focused on investigating the cytotoxicity activity of this plant on Tadpoles (*Ranicepsraninus*). The powdered fruits was extracted with methanol and partitioned with n-hexane, dichloromethane and ethyl acetate. The extract and fractions obtained were evaluated for cytotoxic potential in tadpoles. Bioactive fractions were analyzed on Gas chromatography-Mass spectrometry. The *n*-hexane fraction showed significant cytotoxicity at 20mg/ml, 10mg/ml and 5mg/ml while dichloromethane fraction showed toxicity at 20mg/ml and 10mg/ml. The crude methanol extract and ethyl acetate fraction showed toxicity at 20mg/ml while the aqueous methanol fraction showed no toxicity at all. The GC-MS analysis of *n*-hexane fraction identified Hexadecenoic acid, methyl ester and three (3) other compounds while that of the dichloromethane fraction revealed *n*-hexadecenoic acid and three (3) other compounds. This study justifies the use of *Rauwolfia vomitoria* as an anticancer agent.



**Keywords:***Rauwolfia vomitoria,* Cytotoxicity, fruits, GC-MS analysis


## 1. Introduction

Cancer (neoplasm) is a class of diseases in which a group of cells display uncontrolled or abnormal growth through division beyond normal limit, invasion intrudes upon and destroys adjacent tissues and sometimes metastasis occur, which spreads to cells in other locations in the body via the blood or lymph. Cancers are classified by the type of cell that resembles the tumor and therefore the tissue it's presumed to be the origin of the tumor. These are the histology and the location respectively. They include: Carcinomas, Sarcomas, Leukemia, Lymphoma and Blastoma [1]. The majority of cancers, some 90–95% of cases, are due to genetic mutations from environmental and lifestyle factors [2]. The remaining 5–10% are due to inherited genetics. Environmental refers to any cause that is not inherited, such as lifestyle, economic, and behavioral factors and not merely pollution [3]. Common environmental factors that contribute

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to cancer death include tobacco use (25–30%), diet and obesity (30–35%), infections (15–20%), radiation (both ionizing and non-ionizing, up to 10%), lack of physical activity, and pollution [4]. Psychological stress does not appear to be a risk factor for the onset of cancer, though it may worsen outcomes in those who already have cancer [5].

Cancer is the second leading cause of death globally, accounting for an estimated 9.6 million deaths, or 1in 6 deaths, in 2018. Lung, prostate, colorectal, stomach and liver cancer are the most common types of cancer in men, while breast, colorectal, lung, cervical and thyroid cancer are the most common among women [6]. Globally, the number of cancer deaths is projected to increase from 7.1 million in 2002 to 11.5 million in 2030 [7]. Although, great advancements have been made in the treatment and control of cancer progression, significant deficiencies and room for improvement remain.

Cytotoxicity is the quality of being toxic to cells. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis [8]. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death (apoptosis). Assessing cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects. Cytotoxicity assays measure the ability of cytotoxic compounds to cause cell damage or cell death. Cytotoxicity assays are widely used by the pharmaceutical industry to screen for products that can be eventually be used for treatment of multiline cell pathology and consequently development of anticancer drug [9].

Thus,*Rauvolfia* (sometimes spelled *Rauwolfia*) is a genus of evergreen trees and shrubs, commonly known as devil peppers, in the family Apocynaceae. The genus is named to honor Leonhard Rauwolf. The genus can mainly be found in tropical regions of Africa, Asia, Latin America, and various oceanic island[10]. Herbal preparations of *Rauwolfia vomitoria*, a tropical

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shrub in the family of Apocynaceae, have been used in traditional folk medicine in Africa to treat a variety of ailments including fever, general weakness, gastrointestinal diseases, liver diseases, psychosis, pain, and cancers [11,12].Pharmacological activities including anti-inflammatory, antioxidant[13], antinociceptive [14], cytotoxicity [15], antihypertensive [16],aphrodisiac [17,18] and suppression of prostatic hyperplasia [19]of *R. vomitoria* have been reported. Interestingly, a number of compounds including reserpine, rescinnamine, ajmaline, serpentine rauwolfine, steroid-saposterol and saponins have been reportedly isolated from the leaves, roots and barks [20] but none has been reported from the fruits. Certain herbal preparations used in the treatment of cancer claimed to include *R. vomitoria* fruits. In view of thisinvestigations have to be carried out to assess the potential of the fruits in the killing of cancerous cells. Consequently, this study was aimed at investigating the cytotoxic activity of the fruits of *Rauwolfia vomitoria* using Tadpoles (*Ranicepsraninus*).

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

The fruits of *Rauwolfia vomitoria* was purchased from Oja Oba Market, Ilorin, Kwara State. The fruit was identified and authentiated in the herbarium of the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences. University of Port-Harcourt with a voucher number UPHA0625 assigned. The fruits were picked and separated from twigs. The fruit was then milled to powdered form and kept in an airtight container for further analysis.

### 2.2 Extraction of the fruits of *Rauwolfia vomitoria*

About 450g of the powder was macerated with 2L of methanol for 72h. The extract was filtered and concentrated on a rotary evaporator at 40ºC.

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### 2.3 Partitioning process

About 50g of the methanol extract was partitioned with the following solvents: N-hexane, Dichloromethane, and Ethyl Acetate successively. This partition was done by dissolving 50g of the crude methanol extract with 100ml of ethanol and 200mlof water and placed in a separating funnel. After which, one (1) liter of the N-hexane solvent was added (in 4 parts; 250ml). The mixture was allowed to stand for 5 minutes, then the hexane fractionwas collected and concentrated. The aqueous methanol fraction was further partitioned with dichloromethane and ethyl acetate respective as done for *n*-hexane and each of the fractions were concentrated.

### 2.4 Determination of Cytotoxicity of Extract

Tadpoles were harvested from small water settlements around the areas of Alakahia, Rivers State. They were identified as the tadpoles of *Ranicepsraninus*and used accordingly [21].Five (5) tadpoles were obtained and placed in wide mouthed jars and 15ml of water from their source was added and volume was made up to 50ml. Then, 1ml of the respective concentrations of the crude methanol extract that was dissolved with 2% dimethyl sulfoxide to give the following concentrations; 20mg/ml, 10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml, was added to the corresponding labelled concentration jars containing the tadpoles. This procedure was repeated using the *n*-hexane, Dichloromethane, Ethyl acetate and aqueous methanol fractions. The controls were not treated. The mortality rate of the tadpoles was observed for 24 hours.

### 2.5 Gas Chromatography-Mass Spectrometry (GC-MS)

For the determination of the constituents of the fruits, the *n*-hexane and Dichloromethane fractions were used.On an Agilent chromatograph, coupled to a mass spectrometer equipped with DB DB-IMS capillary column, programmed from 120°C (5min) to 250°C at 3°C/min with 5

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minutes hold time, Helium was used as the carrier gas (1.0ml/min) with a sample injection in split mode (50:1). Injector and detector temperatures were 250-280°C respectively. The mass spectrometer worked in electron impact mode at 70eV with electron multiplier at 1600V and ion source temperature at 180°C. Mass spectra data were acquired in the scan mode range 50-550 m/z. The compounds characterized in the extracts were identified.

## 3. RESULTS AND DISCUSSION

**Table 1:Percentage Yield of the extracts from *Rauwolfia vomitoria***

| SAMPLES | % YIELD |
|---|---|
| Crude methanol extract | 17.44% |
| *n*-hexane fraction | 1.375% |
| Dichloromethane fraction | 0.195% |
| Ethyl acetate fraction | 0.133% |
| Aqueous methanol fraction | 4.200% |

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**Table 2: Toxicity Effect of thefractions of *Rauwolfia vomitoria* fruits on Tadpoles (*Ranicepsraninus*)**

| EXTRACTS | CONCENTRATION | | | | | |
|---|---|---|---|---|---|---|
| | 20mg/ml | 10mg/ml | 5mg/ml | 2.5mg/ml | 1.25mg/ml | |
| Crude methanol extract | 5/5 | 0/5 | 0/5 | 0/5 | 0/5 | |
| *n*-Hexane fraction | 5/5 | 5/5 | 5/5 | 0/5 | 0/5 | |
| Dichloromethane fraction | 5/5 | 5/5 | 0/5 | 0/5 | 0/5 | |
| Ethyl acetate fraction | 3/5 | 0/5 | 0/5 | 0/5 | 0/5 | |
| Aqueous methanol fraction | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | |
| Distilled water | | | | | | 0/5 |
| 2% DMSO | | | | | | 0/5 |

CONTROL

Numerator = Number of deaths; Denominator = Number of tadpoles

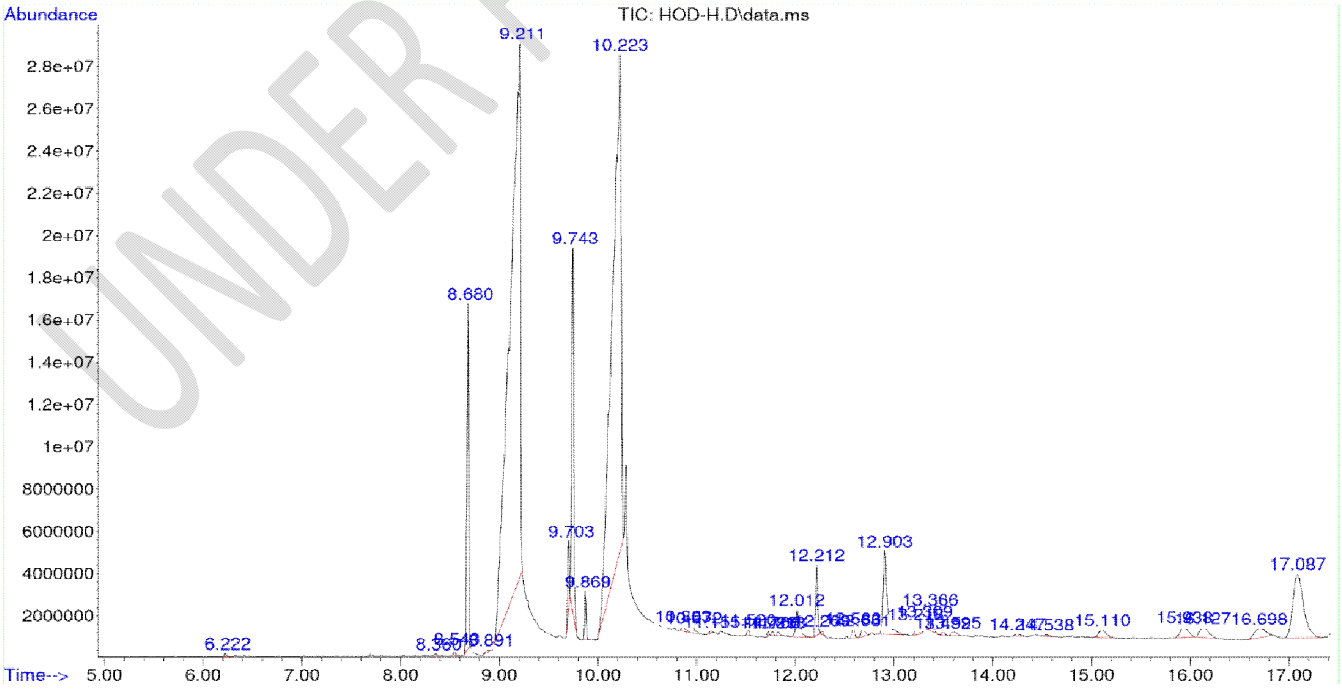


**Figure1: GC-MS Spectra (Chromatogram) of *n*-hexane fraction of *Rauwolfia vomitoria***

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| S/No | Retention time | Area% | Compounds (library ID) | Quality factor |
|---|---|---|---|---|
| 1 | 8.680 | 4.61 | Hexadecenoic acid, methyl ester | 99 |
| 2 | 9.211 | 42.04 | *n*-Hexadecenoic acid | 99 |
| 3 | 9.743 | 5.22 | 9-Octadecenoic acid, methyl ester, (E | 99 |
| 4 | 10.223 | 32.33 | 6-Octadecenoic acid | 99 |
| 5 | 17.087 | 4.94 | Beta- Amyrin | 99 |

**Table 3: Most abundant compounds in the GC-MS Chromatogram of *n*-hexane fraction of *Rauwolfia vomitoria***

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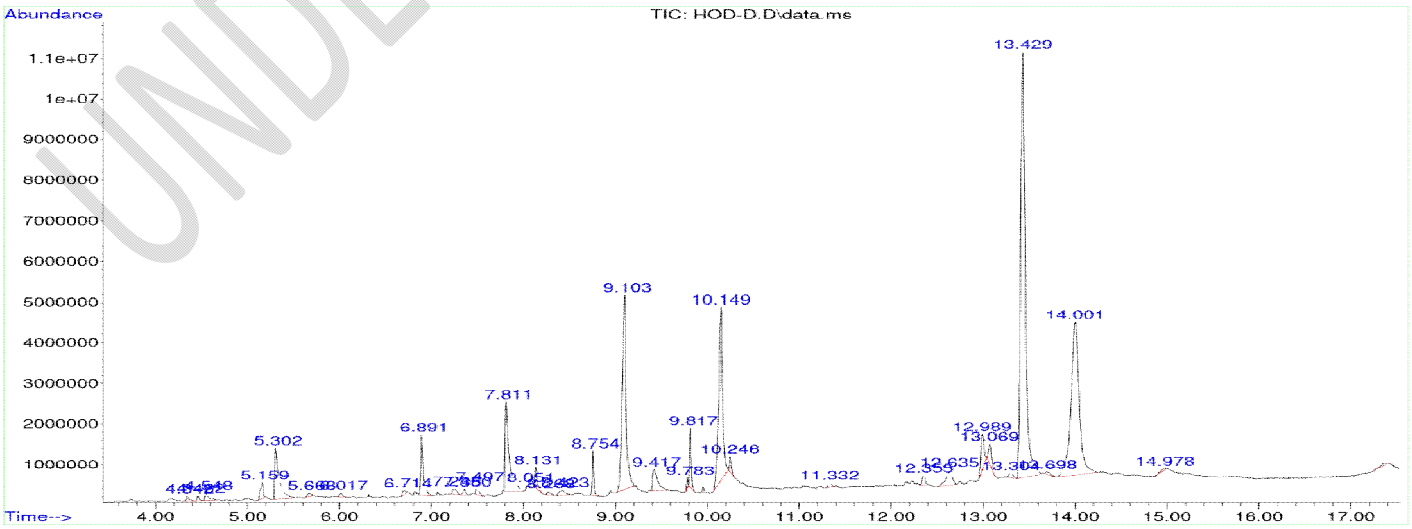

**Figure2: GC-MS Spectra (Chromatogram) of Dichloromethane fraction of *Rauwolfia vomitoria***

**Table 4: Most abundant compounds in the GC-MS Chromatogram of Dichloromethane fraction of *Rauwolfia vomitoria***

| S/NO | RETENTION TIME | AREA % | COMPOUNDS (LIBRARY ID) | QUALITY FACTOR |
|---|---|---|---|---|
| 1 | 9.103 | 11.45 | *n*-Hexadecenoic acid | 99 |
| 2 | 10.149 | 10.61 | 9-Octadecenoic acid | 99 |
| 3 | 13.429 | 31.54 | Ethyl 3,7,11,15-tetramethyl-2-hexadecenoate | 90 |
| 4 | 14.001 | 19.71 | Sarpagan-16-carboxylic acid, 17-oxo, methyl ester | 83 |

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The essence of this research work was to evaluate the cytotoxic activity of *Rauwolfia vomitoria* fruits. This study focuses on the cytotoxic activity of this drug using tadpoles of species *Ranicepsraninus*as a test model. This is because tad poles are developing organisms and are particularly sensitive to various environmental toxins. The percentage yield of the fruit extract and fractionsas presented in Table 1 shows that the fruits contain more polar constituent as the aqueous fraction tends to be higher in percentage (4.2%) when compare to other extractive solvents.

In the determination of the cytotoxic activity of the extract and fractions, It was observed from Table 4that at the end of the 24-h period, the *n*-hexane fraction exhibited the most cytotoxic activity at 5mg/ml, 10mg/ml and 20mg/ml while dichloromethane fraction showed total death at 10mg/ml and 20mg/ml. The aqueous fraction showed no death of tadpoles meaning that it has no cytotoxic effect and unfortunately highest in yield. The methanol extract only showed a total death at the highest concentration 20mg/ml. The ethyl acetate fraction showed mild cytotoxic effect at the highest concentration 20mg/ml with 3 deaths recorded.

The cytotoxicity of the *n*-hexane fraction is in tandem with the reported activity of one of the most abundant compounds (9-Octadecenoic acid, methyl ester (E)) identified by the GC-MS [22]. This is thesame as the dichloromethane fraction which also has a cytotoxic effect due to its relation with one of the most abundant compounds, 9-Octadecenoic acid. It could also be suggested that the most abundant compound in the *n*-hexane (9-Octadecenoic acid, methyl ester (E)) that has cytotoxic effect or activity needs to be further investigated.

Gas chromatography-Mass spectroscopy is a method that is very important in the identification and detection of compounds. It is employed in analysis as it separates compounds in a sample, qualify these compounds and identify unknown peaks. From the GC-MS analysis results of the *n*-hexane fraction, the chromatogram revealed 35 compounds out of which 5 were selected due to their relative abundance in the fraction and their reported activities. The selected compounds were Hexadecenoic acid methyl ester, *n*-Hexadecenoic acid, 9-Octadecenoic methyl ester (E), 6-Octadecenoic acid and beta-Amyrin at the respective times 8.680, 9.211, 9.743, 10.223 and 17.087 with a quality factor of 99 for each. These compounds have varying reported biological activity such as Anti-oxidant, Anti-inflammatory, Anti-bacterial, Anti-fungal, Cancer preventive, Cardio protective, Antinociceptive,Gastro protective and Hepatoprotective activities [23,24,25].

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From the GC-MS analysis of the dichloromethane fraction, the chromatogram revealed about 35 compounds out of which 4 were selected due to their relative abundance. The selected compounds were identified by the Library ID to be; hexadecenoic acid methyl ester, 9-Octadecenoic acid, Ethyl 3, 7, 11, 15- tetramethyl-2-hexadecenoate and Sarpagan-16-carboxylic acid, 17-ox at the respective retention times 9.103, 10.149, 13.429 and 14.001 with quality factors of 99, 99, 90 and 83 respectively. These compounds have varying activities as stated earlier.

## CONCLUSION

In conclusion, the present study suggests that the*n*-hexane fraction and dichloromethane fraction of *Rauwolfia vomitoria*fruits exhibited remarkable cytotoxic activity in the tadpoles implicating Hexadecenoic acid methyl ester, *n*-Hexadecenoic acid, 9-Octadecenoic methyl ester (E), 9 and 6-Octadecenoic acid as the compounds responsible for the activity. The claim that *R. vomitoria* possess cytotoxic activity could be justified in this study.

Disclaimer (Artificial intelligence)

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.

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## REFERENCES

[1] Yadav R. Cancer prevention and control: A review. Journal of Medical Education, 2012;56(1), 1-9.

[2] Anand P,Kunnumakara AB, Sundaram C. Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical Research 2008;25(9), 2097-2116.

[3] Manton KG, Gu X, Lowrimore GR. Cohort changes in active life expectancy in the U.S. elderly population: experience from the 1982–2004 National Long-Term Care Survey. The Journals of Gerontology: Series B, Psychological Sciences and Social Sciences, 2008;63(5), S269–S281.

[4] Islami F, Goding SA, Miller KD. Proportion and number of cancer cases and deaths attributable to potentially modifiable risk factors in the United States. CA CancerJournal of Clinical science, 2018;68(6), 31-54.

[5] Cohen S, Murphy ML, Prather AA. "Ten Surprising Facts About Stressful Life Events and Disease Risk". Annual Review of Psychology. 2019;70, 577–597.

[6] World Health Organization. 2023 progress report on the Global Action Plan for Healthy Lives and Well-being for All.

[7] Mathers CD, Loncar D, Lopez AD, & Murray, C.J.L. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Medicine, 2006;3(11), e442.

[8] Vinken M &Blaauber BJ. In vitro testing of basal cytotoxicity: establishment of an adverse outcome pathway from chemical insult to cell death. Toxicology in Vitro. 2017; 39:104-110. DOI:10.1016/j.tiv.2016.12.004

[9] Teng, X., & Yang, Z. (2014). "Cytotoxicity of natural products and synthetic compounds." Journal of Applied Toxicology, 34(5), 508-520.

[10] FamiliesJ. Rauvolfia: A review of its medicinal uses and safety aspects. Journal of Ethnopharmacology, 2014;133(2), 402-411.

[11] Campbell-Tofte JIA, Mølgaard P, Josefsen K., et al. Randomized and double-blinded pilot clinical study of the safety and anti-diabetic efficacy of the Rauvolfia-Citrus tea, as used in Nigerian traditional medicine. Journal of Ethnopharmacology. 2011;133(2), 402-411.

[12] Bisong SA, Brown RE, Osim EE. Comparative extrapyramidal effects of *Rauwolfia vomitoria*, chlorpromazine and reserpine in mice. Journal of Natural Medicines.2013;67, 107-112.

[13] Youmbie DDB, Dzeufiet DPD, Nkwengoua ZE,Zingue S, Mezui C, Bibi FO, Tankeu NF, Pieme CA, Dimo T. Anti-inflammatory and anti-oxidant effects of the stem bark

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Aqueous extract of Rauwolfiavomitoria (apocynaceae) in female Wistar rats. European Journal of Pharmaceutical and Medical Research 2015;2(7), 64-73

[14] Youmbie DDB, Dzeufiet DPD, Nkwengoua E, Mezui C, Dimo T. Anti-inflammatory and antinociceptive effects of the stem bark aqueous extract of Rauwolfia vomitoria. World J Pharm Pharma Sci. 2015;4: 90-112

[15] Abdelfatah SA, Efferth T. Cytotoxicity of the indole alkaloid reserpine from Rauwolfiaserpentina against drug-resistant tumor cells. Phytomedicine. 2015 Feb 15;22(2):308-18.

[16] Robert W. Wilkins, Rauwolfia in the treatment of essential hypertension, The American Journal of Medicine, Volume 17, Issue 5, 1954, Pages 703-711, ISSN 0002-9343, https://doi.org/10.1016/0002-9343(54)90029-7. (https://www.sciencedirect.com/science/article/pii/0002934354900297)

[17] Koloko BL, Bushra I, Wankeu-Nya M, et al. In vivo effects of Rauvolfiavomitoria (Apocynaceae) ethanolic extract on sexual performance and reproductive activity in male rats. Andrologia. 2020; 52:e13414. https://doi.org/10.1111/and.13414

[18] Kada AS, Aneng E, Mbouh M, Manfo FPT, Ngimgoh SN, Nantia EA. Testicular antioxidants and testosterone enhancing effects of the hydroethanolic extract of Rauvolfiavomitoria (Apocynacaea) in male Wistar rats. Investigational Medicinal Chemistry and Pharmacology. 2021;4(2):54; Doi: https://dx.doi.org/10.31183/imcp.2021.00054

[19] Huang G, He X, Xue Z. et al. Rauwolfia vomitoria extract suppresses benign prostatic hyperplasia by inducing autophagic apoptosis through endoplasmic reticulum stress. BMC Complement Med Ther 22, 125 (2022). https://doi.org/10.1186/s12906-022-03610-4

[20] Kumar S, Kumari D, Singh B. Genus Rauvolfia: A review of its ethnopharmacology, phytochemistry, quality control/quality assurance, pharmacological activities and clinical evidence. J Ethnopharmacol. 2022 Sep 15;295:115327. doi: 10.1016/j.jep.2022.115327. Epub 2022 Apr 30. PMID: 35504505.

[21] Yu F, Lu S, Yu F, Shi J, McGuire PM, Wang R. Cytotoxic activity of an octadecenoic acid extract from *Euphorbia kansui* (Euphorbiaceae) on human tumour cell strains. J Pharm Pharmacol. 2008 Feb;60(2):253-9. doi: 10.1211/jpp.60.2.0015. PMID: 18237474.

[22] Ikpefan EO, Ayinde BA. Preliminary Phytochemical and Cytotoxic Activity of The Aqueous and Chloroform Fractions of The Leaf of *Persea Americana* Mill (Lauraceae).

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NIJOPHASR [Internet]. 2020 Aug. 2 [cited 2024 Dec. 25];5(1):15-20. Available from: https://www.nijophasr.net/index.php/nijophasr/article/view/107

[23] Zhong-hui PU, Yu-qun ZHANG, Zhong-qiong YIN, Jiao XU, Ren-yong JIA, Yang LU, Fan YANG. Antibacterial Activity of 9-Octadecanoic Acid-Hexadecanoic Acid-Tetrahydrofuran-3,4-Diyl Ester from Neem Oil, Agricultural Sciences in China. 2010;9(8):1236-1240,

[24] Waqas A, Imad A, Mubarika A, Faizullah K, Haroon K, Chapter 8 - Neuroprotective effect of terpenoids, Editor(s): Haroon K, Michael A, Hamed M,Phytonutrients and Neurological Disorders, Academic Press, 2023;227-244, ISBN 9780128244678, https://doi.org/10.1016/B978-0-12-824467-8.00006-1.

[25] Bhardwaj R. GC-MS analysis and antimicrobial activity of alkaloids of Tecomellaundulata. J Med Plant Stud. 2018; 6(6):68–72.

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