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BIOACTIVE, ANTIOXIDANT AND GAS CHROMATOGRAPHY-MASS SPECTROMETER ANALYSES OF ZANTHOXYLUM ZANTHOXYLOIDES SHOOT

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Abstract

Zanthozylum zanthozyloides is a widely distributed plant that possesses numerous medicinal properties. This study was designed to assess the phytochemical constituents, antioxidant property, and cytotoxicity of flavonoid- rich extracts of shoot of Z. zanthozyloides. The crude ethanolic extract of the tested plant was screened for bioactive compounds and the flavonoids-rich extract was obtained. The plant extracts were subjected to 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) and hydrogen peroxide (H_2O_2) radicals scavenging activity, and brine shrimp lethality (BSL) assay in comparison to standard substances. The bio-active compounds in the crude extract were determined via gas liquid chromatography-mass spectroscopy (GC-MS). Flavonoids, alkaloids, saponins, tannins, glycosides, steroids and carbohydrates were detected in the crude extract. Likewise, compounds like squalene, vitamin E, 3-carene and limonene were present in varied concentrations. The extracts caused a dose-dependent antioxidant and BSL cytotoxic activities. In all the antioxidant evaluations, flavonoid-rich extract significantly ($p<0.05$) scavenged DPPH, NO and H_2O_2 radicals than the crude extract, but lower than the ascorbic acid. Moreover, the flavonoid extract caused a serious mortality at 85.52 µg/ mL lethal concentration on 50% of the nauplii population (LC_{50}). This activity was insufficiently ($p<0.05$) effective as the doxorubicin (7.62 µg/ mL), but significantly ($p<0.05$) more than the crude extract (99.58 µg/ mL). These results corroborate the presence of bioactive volatile compounds with potential antiradical and cytotoxic activity in the studied plant sample.

Keywords: *Z. zanthoxyloides shoot extract, cytotoxicity, antioxidants, bioactive compounds.*

1. Introduction

Natural products are secondary metabolites that an organism produces; many of these metabolites are unique to that organism. Secondary metabolites perform no explicit involvement in the organism's internal economy. Organisms use them for protective or adaptive purposes (Yadav *et al.,* 2019). Natural products are found in different facet of life including plant, animals and microorganisms. Plant-based natural products are one of the bases for the development of new pharmaceuticals that are more effective, safer, and pharmacologically superior than synthetic drugs (Andrade *et al.,* 2018). Bioactive chemicals found in plants are responsible for their health-promoting properties (KossMikoajczyk *et al.,* 2019). They showed biological effects against chronic illnesses in several investigations, which consequently account for their use in alternative or supplemental therapy; particularly in developing countries (Ayeleso *et al.,* 2017; Ozioma and Chinwe, 2019). Medicinal properties of plants and herbal components include anti-cancer, antiglycemic, anti-inflamatory, antihyperglycemic and analgesic properties in studies (Bouyahya *et al.,* 2021; Davoodvandi *et al.,* 2019; Rana *et al.,* 2021). Moreover, many of these biological activities are derived from the antioxidant ability of the plant derived natural products which resultantly lessens cellular oxidative damages in other to avert related diseases like cancer (Yang *et al.,* 2019). These natural products include alkaloids, flavonoids, lignans, taxanes, vitamins, minerals, gums, oils, biomolecules e.t.c., and are used alone or in combination during chemotherapy (Shamran and Abed, 2020; Bose *et al.,* 2020).

Z. zanthoxyloides (Rutaceae) is commonly found across a variety of habitats, and is used to treat indigestion, toothaches, abdominal pain, sickle cell anemia, bacterial and cancer e.t.c. (Guendéhou *et al.,* 2018; Sado Kamdem *et al.,* 2015). Assessment of bioactive compounds for biological activities can be done by probing their antioxidant or cytotoxic property by use of *in vitro* or *in vivo* model (Saleem *et al*., 2019). Antioxidant of flavonoids in *Z. zanthoxyloides* could be assessed by probing its effect on highly reactive unstable nitrogen or oxygen species. In other cases, toxicity to normal or diseased cells can be investigated by use of cytotoxicity measurement in human cell lines or animal cells (newly hatched brine shrimps) in *in vitro* or *in vivo* experiments, and the bioactive components characterized by the use of chromatographic experiments such as the gas liquid chromatography (Sarah *et al.,* 2018; Ishtiaq *et al*., 2020). Thus, the goal of this study was to determine the biological activity of *Z. zanthozyloides* by investigating its antiradical and cytotoxic activities, and authenticate the plant chemicals using GC-MS.

2. Materials and Methods

2.1 Plant Materials

Sample of matured shoot of *Z. zanthozyloides* was collected from a forest in Oyo town, Oyo State, Nigeria, in the month of November and voucher specimen was submitted for authentication (LUH 6909) in the herbarium of the University of Lagos, Akoka. A portion of the plant sample was gently rinsed with tap water and dried on the laboratory bench before grinding to coarse powder in the laboratory mortal and reserved for extraction.

2.2 Preparation of crude extract of *Z. zanthozyloides*

A total of 800 grams of the ground sample were measured and extracted for 48 hours in 60 percent ethanol. The resulting filtrate was then concentrated to a paste (25.47±0.03% crude extract) using a rotary evaporator after filtration with Whatman No. 42 filter paper. The crude extract was screened for phytochemicals, while the other portion was kept for future use.

2.3 Phytochemical screening of crude extract of *Z. zanthozyloides*

The presence of the bioactive compounds was determined in the crude extract by conventional methods published by Harbone (1973), Sofowora (1993) and Evans (1993).

2.4 Extraction of flavonoids from *Z. zanthozyloides*

The extraction and separation of flavonoids from *Z. zanthozyloides* shoots were carried out using the method of (Lee *et al.,* 1995). The flavonoid residue (6.70 ± 0.07%) was left after evaporating the organic layer at 40°C.

2.5 Antioxidant activity of extracts of *Z. zanthozyloides*

Method of Joshi *et al.* (2015) was used to determine the DPPH radical scavenging activity, the scavenging of NO generated from sodium nitroprusside was assessed according to Nanyonga *et al.* (2013). The scavenging activity was also determined (Ngonda, 2013). The experiment was conducted in triplicates and the radical scavenging activity calculated as in the equation below.

$$\%\ Scavenging\ activity = \frac{(absorbance\ of\ control - absorbance\ of\ sample)100}{absorbance\ of\ control}$$

2.6 BSL assay of extract of *Z. zanthozyloides*

The protocol of Meyer *et al.* (Meyer *et al.*, 1982) was adopted with minor changes. Using a transparent Pasteur pipette, ten (10) active nauplii were selected and subjected to treatment with 1 mL of various quantities of fractions of *Z. zanthozyloides* in DMSO for 24 hours in test tubes to. With clean seawater, the volume was increased to 10 mL. After 24 hours, active nauplii were counted, and percentage mortality was calculated. For each of the sample concentrations, the experiment was repeated three times. Using probit analysis and IBM SPSS Statistics 20 software, the lethal concentration that killed 50% of the nauplii population (LC_{50}) was calculated.

2.7 Preparation of plant the extract for GC-MS analysis

Considering the results from the investigated biological analyzes, 1g/ mL solution of ethanol extract of *Z. zanthozyloides* was prepared in concentrated ethanol (HPLC grade) and filtered with Whatman #1 filter paper before subjecting it to the GC-MS analysis 24 hours after then. Polar and non-polar phytocomponents in the ethanol extract of *Z. zanthozyloides* was determined in GC-MS. The analysis was performed on a Perkin Elmer GC Clarus 500 system that included an AOC-20i autosampler and a gas chromatograph linked to a mass spectrometer comprising of Elite -1 column - Fused silica capillary column (30 x 0.25 mm ID x 1 df, made entirely of 100% dimethylpolysiloxane) working at 70 eV in electron impact mode. At a continuous flow of 1 mL/min and an injection volume of 0.5 L (split ratio of 10:1), helium gas (99.999 percent) was employed as the carrier gas while the injector temperature of 250°C. The temperature of the ion source for the detection was 280°C. The oven temperature maintained at isothermal temperature of 110°C for 2 minutes with a steady increase of 10°C until it rose to 200°C, this was followed by a rise 5°C/min to 280°C and finally with a 9 minutes isothermal at 280°C. The identification of the mass spectrum obtained from the GC-MS was performed using the National Institute Standard and Technology (NIST) by comparing the spectrum of the unknown component with the known components contained in the NIST database, and the name, molecular weight and structure of the phyto-components of the understudy plant were determined.

2.8 Statistical analysis

Dunnett's post hoc test using GraphPad Prism software was used to analyze and gather all analytical data, graphs, and significant differences between treatments.

3. Results and Discussion

Table 1: Bioactive compounds in ethanol extract of *Z. zanthozyloides.*

Phytochemicals	Phenolics	Flavonoids	Alkaloids	Saponins	Tannins	Glycosides	Steroids	Carbohydrates
Crude extract	+	+	+	+	+	+	+	+

Where (+ve) indicates that the compound was detectable.

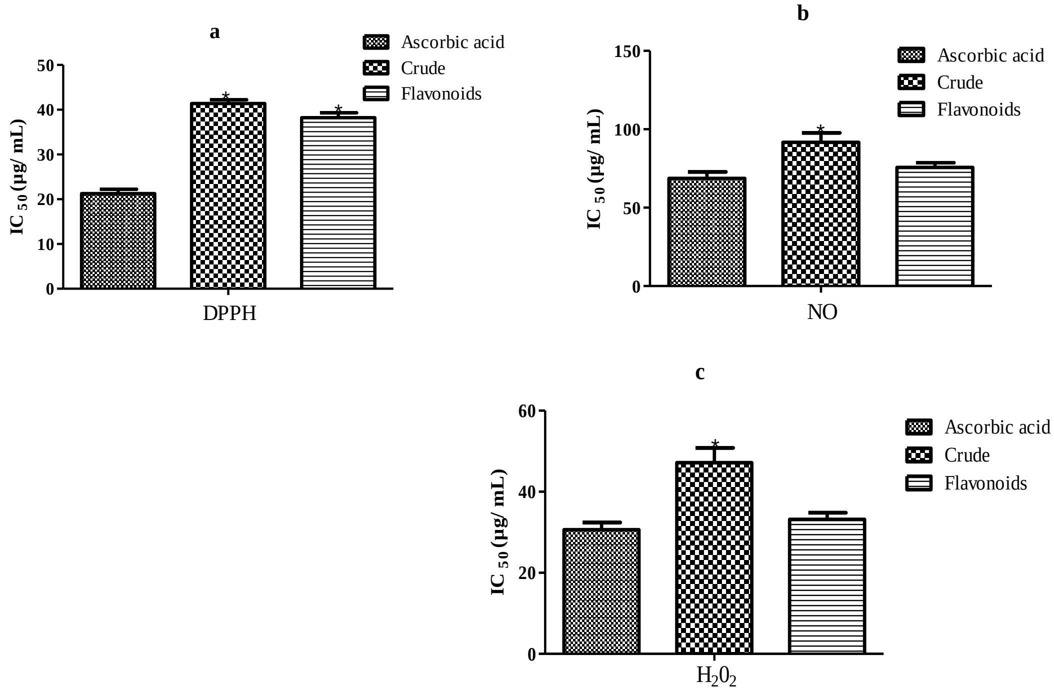


Figure 1: Free radical scavenging activity of *Z. zanthozyloides*.

Data are represented in terms of mean ± SEM of triplicate readings. The (*) implies a significant difference at ($p<0.05$) when compared with ascorbic acid.

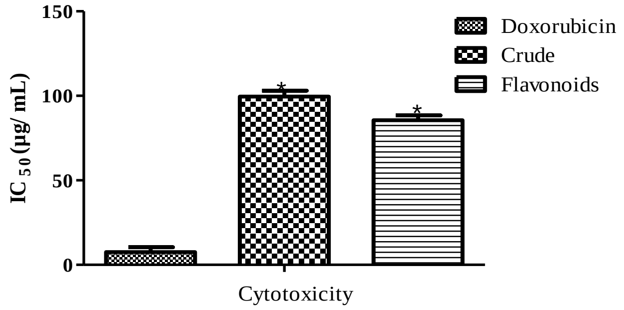
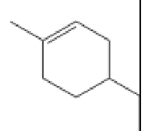
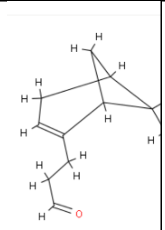
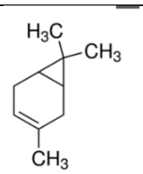
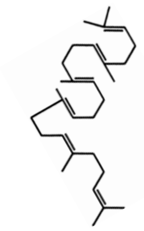
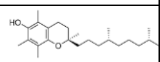
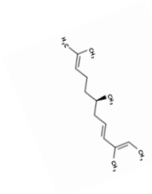
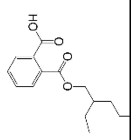
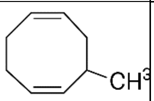


Figure 2: Cytotoxic activity of *Z. zanthozyloides*.

Data are represented in terms of mean ± SEM of triplicate readings. The (*) implies a significant difference at ($p<0.05$) when compared with Doxorubicin.

Table 2: GC-MS analysis of ethanolic extract of *Z. zanthozyloides*.

RT	% area	Compound	Molecular formula	Molecular mass	Structure	Biological activities	References
6.686	6.65	Limonene	$C_{10}H_{16}$	136.238		Anticancer/ antitumor by apoptosis, antibacterial, antioxidant, anti-inflamatory, antidiabetic,	(Anandakumar *et al.*, 2021; Costa *et al.*, 2019; Vieira *et al.*, 2018; Araújo-Filho *et al.*, 2021)

						gastroprotective and antiviral.	
7.155	0.72	3-(1S,5S,6R)-2,6-Dimethylbicyclo[3.1.1]hept-2-3n-6-yl) propanal	$C_{12}H_{18}$	178.271		Antiperspirant and deodorant	(Duke, 1992)
7.155	0.72	3-Carene	$C_{10}H_{16}$	136.24		Antifungal and antimicrobial.	(Shu *et al.*, 2019; Kang *et al.*, 2019)
31.124	1.52	Squalene	$C_{30}H_{50}$	410.73		Antibacterial, Antioxidant, Antitumor, Cancer-Preventive, Chemopreventive, Immunostimulant.	Kim and Karadeni, 2012; Rajamani *et al.*, 2021; Yakubogullari *et al.*, 2021).
33.659	3.52	Vitamin E	$C_{29}H_{50}O_2$	430.71		Analgesic, anti-inflammatory, antioxidant, antitumor, vasodilator, and antileukemic.	(Salma *et al.*, 2018; Perumpail *et al.*, 2018; (Abu-Fayyad *et al.*, 2017)
29.83	8.52	Caparratriene	$C_{15}H_{26}$	206.4		Antileukemia	(Vydrina *et al.*, 2018)
28.68		Mono(2-ethylhexyl) phthalate	$C_{16}H_{22}O_4$	278.4		Spermatogenic apoptosis	(Fu *et al.*, 2018; Bahrami *et al.*, 2018)
29.56		1,5-Cyclooctadiene, 3-methyl-	C_9H_{14}	122.2		Cytotoxic	(Daubit *et al.*, 2021)

The percentage extraction yields of the crude extract of *Z. zanthoxyloides* (25.47±0.03 µg/ mL) varied with the flavonoid-rich extract (6.70±0.07 µg/ mL) indicating the presence other compounds which were sequentially removed in the course of extraction of flavonoids in the selected plant. Screening for biologically active compounds in medicinal plants is important in studying the pharmacological activity of such plants. Thus, qualitative screening of crude *Z. zanthozyloides* extract revealed the detection of compounds such as alkaloids, flavonoids, phenols, tannins, glycosides saponins, and carbohydrates with the exclusion of steroids (Table 1). This has been previously corroborated by previous study (Olusola *et al.*, 2020). These compounds are relevant in the protection of plant and animal health; releasing odors and repelling substances against pests (Ajuru *et al.*, 2017; Ogbonna *et al.*, 2018). They also showed antioxidant, antimicrobial, anti-inflammatory, wounds healing, anticancer/antitumor, and cytotoxic effects (Al Ayash, 2020; Karak, 2019; Ghosh *et al.*, 2019; Shahzad *et al.*, 2020; Tanase *et al.*, 2019).

The mode of antioxidant activity of *Z. zanthozyloides* was demonstrated by spectrophotometric assays using DPPH, NO and H_2O_2 scavenging activities (Figure 1). Usually, a lower IC_{50} value indicates greater antioxidant activity. Ascorbic acid was used as reference antioxidant for the assays. The ability of the flavonoid extract to scavenge free radicals from

DPPH, NO or H_2O_2 was significantly (*$p<0.05$*) lower than the ascorbic acid, but higher than the crude extract. In the DPPH scavenging ability assay, flavonoids had 38.23±1.09 μg/ mL while the crude extract had 41.41±0.81 μg/ mL. Polyhenolics like flavonoids have been reported to possess antioxidant activity (Jakubczyk *et al.*, 2020). Kumar *et al.*, (2020) inferred the DPPH radicals scavenging ability of flavonoids. This test revealed that the *Z. zanthozyloides* changed the violet-colored DPPH to a yellow-colored DPPH (picryl) derivative via releasing electrons or hydrogen radical to the DPPH radical (Habu and Ibe, 2015). NO is a major free radical that is generated in mammalian cells for the maintenance of physiological activities, but may damage organs and tissues, and cause health challenges like cancer and other inflammatory conditions (Habu and Ibe, 2015). The flavonoid extract interacts with the peroxynitrite anion ($ONOO^-$) highly reactive compound (Nagmoti *et al.*, 2012). The potency of polyphenolics like flavonoids as NO scavenger was previously reported (Senguttuvan *et al*., 2014). The extracted bioactive compound in the extract of *Z. zanthozyloides* scavenged H_2O_2 radicals by donating electrons to H_2O_2 and converting the radicals to water (Ali *et al.*, 2021). Thus, mitigating the ability of H_2O_2 to activate the cell proliferation or differentiation signaling pathway in other to prevent oxidative stress and inflammatory responses that cause conditions like cancer and cardiovascular (Lennicke *et al.*, 2015; Nandi *et al.*, 2019).

The plant's biological activity was also established by the *in vitro* cytotoxicity in the newly hatched brine shrimps; a rapid, inexpensive, and simple bioassay with a significant correlation with cytotoxic and antitumor properties (Elmore, 2007; Anderson *et al.*, 1991). This toxic response is usually determined by changes in cell survival or metabolism (McGaw *et al.*, 2014). The results for the BSL assay presented as LC_{50} indicted flavonoid-rich extract (88.52±2.95 μg/ mL) with optimal cytotoxicity and the crude extract (99.58±3.46 μg/ mL) as the least cytotoxic when compared to the doxorubicin (Figure 2). Thus, the cytotoxicity of the extract of *Z. zanthozyloides* was lesser than the doxorubicin. Fatima *et al*. (2015) reported that any plant extracts having LD_{50} lower than 100 μg/ mL should be regarded highly cytotoxic. This report is in accord with the Niksic *et al.* (2021). Braguini *et al.* (2018) also showed that *S. viarum* (LC_{50} = 66.01 μg/ mL) was cytotoxic in active nauplii.

GC-MS analysis of the crude extract reveals the presence of diverse bioactive compounds including limonene, vitamin E (α-tocopherol), squalene, 3-Carene and 3-(1S,5S,6R)-2,6-Dimethylbicyclo [3.1.1] hept-2-3n-6-yl) propanal, whose biological properties include anticancer through induction of apoptosis, antimicrobial, antimutagenic and antiviral, antiperspirant and deodorant, antioxidant, analgesic, anti-inflammatory and cytotoxic activities. Vitamin E is an analgesic, anti-aging, anti-inflammatory, and anti-tumor, antioxidant, anti-proliferative, apoptotic, anticancer agents. Also, limonene possesses anticancer, anti-inflammatory, antitumor, apoptotic, detoxifying, chemo-preventive, antibacterial and anti-mutagenic effects (Yao *et al.*, 2017; Chen *et al.*, 2018).

These polyhenolics (limonene, vitamin E and squalene) in the hydro-ethanolic extract of *Z. zanthoxyloides* might be responsible for the biological properties (antioxidant and cytotoxicity) and may predict its anticancer and antimicrobial properties. Theese compounds are responsible for the traditional use of the plant as antimicrobial or anticancer therapy (Niksic *et al.*, 2021).

4. Conclusion

The *Z. zanthozyloides* contains nutritional and pharmacologic agents that can be administered as supplements, used for treatment or prevention of free radical inciting diseases like microbial attacks, cancer, inflammatory, pains and aging.

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6. REFERENCES

[1] Yadav, A.N., Kour, D., Rana, K.L., Yadav, N., Singh, B., Chauhan, V.S., Rastegari, A.A., Hesham, A.E.L. and Gupta, V.K., 2019. Metabolic engineering to synthetic biology of secondary metabolites production. In *New and future developments in microbial biotechnology and bioengineering* (pp. 279-320). Elsevier.

[2] Andrade, J.M., Faustino, C., Garcia, C., Ladeiras, D., Reis, C.P. and Rijo, P., 2018. Rosmarinus officinalis L.: an update review of its phytochemistry and biological activity. *Future science OA*, *4*(4), p. FSO283.

[3] Koss-Mikołajczyk, I., Kusznierewicz, B., Wiczkowski, W., Płatosz, N., & Bartoszek, A. (2019). Phytochemical composition and biological activities of differently pigmented cabbage (Brassica oleracea var. capitata) and cauliflower (Brassica oleracea var. botrytis) varieties. *Journal of the Science of Food and Agriculture*, *99*(12), 5499-5507.

[4] Ayeleso, T.B., Matumba, M.G. and Mukwevho, E., 2017. Oleanolic acid and its derivatives: biological activities and therapeutic potential in chronic diseases. *Molecules*, *22*(11), p.1915.

[5] Ozioma, E.O.J. and Chinwe, O.A.N., 2019. Herbal medicines in African traditional medicine. *Herbal medicine*, *10*, pp.191-214.

[6] Abu-Fayyad, A. and Nazzal, S., 2017. Gemcitabine-vitamin E conjugates: Synthesis, characterization, entrapment into nanoemulsions, and in-vitro deamination and antitumor activity. International journal of pharmaceutics, 528(1-2), pp.463-470.

[7] Ajuru, M.G., Williams, L.F. and Ajuru, G., 2017. Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta Region of Nigeria. *Journal of Food and Nutrition Sciences*, *5*(5), pp.198-205.

[8] Al Ayash, S. (2020). *Chemical composition, antioxidant and anti-diabetic activities of Scorzonera Phaeopappa Boiss* (Doctoral dissertation, Notre Dame University-Louaize).

[9] Ali, M. N., Ndahi, J. A., Abdullahi, A., & Yelwa, J. M. (2021). Phytochemicals, chemical composition and antioxidants profile of the crude extracts and essential oil of Mitracarpus scaber (Goga masu).

[10] Anandakumar, P., Kamaraj, S. and Vanitha, M.K., 2021. D‐limonene: A multifunctional compound with potent therapeutic effects. *Journal of food biochemistry*, *45*(1), p.e13566.

[11] Anderson, J.E., Goetz, C.M., McLaughlin, J.L. and Suffness, M., 1991. A blind comparison of simple bench‐top bioassays and human tumour cell cytotoxicities as antitumor prescreens. *Phytochemical analysis*, *2*(3), pp.107-111.

[12] Araújo‐Filho, H.G.D., Dos Santos, J.F., Carvalho, M.T., Picot, L., Fruitier‐Arnaudin, I., Groult, H., Quintans‐Júnior, L.J. and Quintans, J.S., 2021. Anticancer activity of limonene: A systematic review of target signaling pathways. *Phytotherapy Research*, *35*(9), pp.4957-4970 Araújo‐Filho, H. G. D., Dos Santos, J. F., Carvalho, M. T., Picot, L., Fruitier‐Arnaudin, I., Groult, H., ... & Quintans, J. S. (2021). Anticancer activity of limonene: A systematic review of target signaling pathways. *Phytotherapy Research*, *35*(9), 4957-4970.

[13] Bahrami, N., Goudarzi, M., Hosseinzadeh, A., Sabbagh, S., Reiter, R.J. and Mehrzadi, S., 2018. Evaluating the protective effects of melatonin on di (2-ethylhexyl) phthalate-induced testicular injury in adult mice. *Biomedicine & Pharmacotherapy*, *108*, pp.515-523.

[14] Bose, S., Banerjee, S., Mondal, A., Chakraborty, U., Pumarol, J., Croley, C.R. and Bishayee, A., 2020. Targeting the JAK/STAT signaling pathway using phytocompounds for cancer prevention and therapy. *Cells*, *9*(6), p.1451.

[15] Bouyahya, A., Guaouguaou, F. E., El Omari, N., El Menyiy, N., Balahbib, A., El-Shazly, M., & Bakri, Y. (2021). Anti-inflammatory and analgesic properties of moroccan medicinal plants: Phytochemistry, in vitro and in vivo investigations, mechanism insights, clinical evidence and perspectives. *Journal of Pharmaceutical Analysis*.

[16] Braguini, W.L., Pires, N.V. and Alves, B.B., 2018. Phytochemical Analysis, Antioxidant Properties and Brine Shrimp Lethality of Unripe Fruits of Solanum viarum. *Journal of Young Pharmacists*, *10*(2).

[17] Chen, Y.W., Huang, P.H., Tsai, Y.H., Jiang, C.M. and Hou, C.Y., 2018. Effects of Limonene on the PAHs mutagenicity risk in Roasted Fish Skin.

[18] Costa, M.D.S., Rocha, J.E., Campina, F.F., Silva, A.R., Da Cruz, R.P., Pereira, R.L., Quintans-Júnior, L.J., De Menezes, I.R., Adriano, A.D.S., De Freitas, T.S. and Teixeira, A.M., 2019. Comparative analysis of the antibacterial and drug-modulatory effect of d-limonene alone and complexed with β-cyclodextrin. *European Journal of Pharmaceutical Sciences*, *128*, pp.158-161.

[19] Daubit, I.M., Wortmann, S., Siegmund, D., Hahn, S., Nuernberger, P. and Metzler‐Nolte, N., 2021. Unveiling Luminescent IrI and RhI N‐Heterocyclic Carbene Complexes: Structure, Photophysical Specifics, and Cellular Localization in the Endoplasmic Reticulum. *Chemistry–A European Journal*, *27*(22), pp.6783-6794.

[20] Davoodvandi, A., Sahebnasagh, R., Mardanshah, O., Asemi, Z., Nejati, M., Shahrzad, M.K., Mirzaei, H.R. and Mirzaei, H., 2019. Medicinal plants as natural polarizers of macrophages: phytochemicals and pharmacological effects. *Current pharmaceutical design*, *25*(30), pp.3225-3238.

[21] Duke, J.A., 1992. *Database of phytochemical constituents of GRAS herbs and other economic plants*. CRC Press.

[22] Elmore, S., 2007. Apoptosis: a review of programmed cell death. *Toxicologic pathology*, *35*(4), pp.495-516.

[23] Evans, W. C. (2009). *Trease and Evans' pharmacognosy*. Elsevier Health Sciences.

[24] Fatima, H., Khan, K., Zia, M., Ur-Rehman, T., Mirza, B., & Haq, I. U. (2015). Extraction optimization of medicinally important metabolites from Datura innoxia Mill.: an in vitro biological and phytochemical investigation. *BMC complementary and alternative medicine*, *15*(1), 1-18.

[25] Fu, Y., Dong, J., Wang, J., You, M., Wei, L., Fu, H., Wang, Y. and Chen, J., 2018. Developmental exposure to di-(2-ethylhexyl) phthalate induces cerebellar granule cell apoptosis via the PI3K/AKT signaling pathway. *Experimental neurobiology*, *27*(6), p.472.

[26] Ghosh, P., Ghosh, C., Das, S., Das, C., Mandal, S. and Chatterjee, S., 2019. Botanical description, phytochemical constituents and pharmacological properties of Euphorbia hirta Linn: a review. *International Journal of Health Sciences and Research*, *9*(3), pp.273-286.

[27] Guendéhou, F., Djossa, B.A., Kènou, C. and Assogbadjo, C.A.E., 2018. Review of studies on *Z. zanthoxyloids* (Lam): Availability and ethnomedical, phytochemical, pharmacological uses. *Schol. J. Res. Agric. Biol*, *3*(3), pp.244-254.

[28] Habu, J. B., & Ibeh, B. O. (2015). In vitro antioxidant capacity and free radical scavenging evaluation of active metabolite constituents of *Newbouldia laevis ethanolic* leaf extract. *Biological Research*, *48*(1), 1-10.

[29] Harbone, J (1973). Methods of plants analysis in: phytochemical methods chapman and Hall, London.

[30] Ishtiaq, S., Hanif, U., Shaheen, S., Bahadur, S., Liaqat, I., Awan, U. F., ... & Meo, M. (2020). Antioxidant potential and chemical characterization of bioactive compounds from a medicinal plant Colebrokea oppositifolia Sm. *Anais da Academia Brasileira de Ciências*, *92*.

[31] Jakubczyk, K., Kałduńska, J., Kochman, J. and Janda, K., 2020. Chemical profile and antioxidant activity of the kombucha beverage derived from white, green, black and red tea. *Antioxidants*, *9*(5), p.447.
[32] Joshi, K.R., Devkota, H.P., Nakamura, T., Watanabe, T. and Yahara, S., 2015. Chemical constituents and their DPPH radical scavenging activity of nepalese crude drug Begonia picta. *Records of Natural Products*, *9*(3), p.446.
[33] Kang, G.Q., Duan, W.G., Lin, G.S., Yu, Y.P., Wang, X.Y. and Lu, S.Z., 2019. Synthesis of bioactive compounds from 3-carene (II): Synthesis, antifungal activity and 3D-QSAR study of (Z)-and (E)-3-caren-5-one oxime sulfonates. *Molecules*, *24*(3), p.477.
[34] Karak, P., 2019. Biological activities of flavonoids: an overview. *Int. J. Pharm. Sci. Res*, *10*(4), pp.1567-1574.
[35] Kim, S.K. and Karadeniz, F., 2012. Biological importance and applications of squalene and squalane. *Advances in food and nutrition research*, *65*, pp.223-233.
[36] Koss-Mikołajczyk, I., Kusznierewicz, B., Wiczkowski, W., Sawicki, T., & Bartoszek, A. (2019). The comparison of betalain composition and chosen biological activities for differently pigmented prickly pear (Opuntia ficus-indica) and beetroot (Beta vulgaris) varieties. *International Journal of Food Sciences and Nutrition*, *70*(4), 442-452.
[37] Kumar, A., Mahajan, A. and Begum, Z., 2020. Phytochemical screening and in vitro study of free radical scavenging activity of flavonoids of aloe vera. *Research Journal of Pharmacy and Technology*, *13*(2), pp.593-598.
[38] Lennicke, C., Rahn, J., Lichtenfels, R., Wessjohann, L.A. and Seliger, B., 2015. Hydrogen peroxide–production, fate and role in redox signaling of tumor cells. *Cell Communication and Signaling*, *13*(1), pp.1-19.
[39] McGaw, L. J., Elgorashi, E. E., & Eloff, J. N. (2014). Cytotoxicity of African medicinal plants against normal animal and human cells. In *Toxicological survey of African medicinal plants* (pp. 181-233). Elsevier.
[40] Nagmoti, D.M., Khatri, D.K., Juvekar, P.R. and Juvekar, A.R., 2012. Antioxidant activity free radical-scavenging potential of Pithecellobium dulce Benth seed extracts. *Free Radicals and Antioxidants*, *2*(2), pp.37-43.
[41] Nandi, A., Yan, L.J., Jana, C.K. and Das, N., 2019. Role of catalase in oxidative stress-and age-associated degenerative diseases. *Oxidative medicine and cellular longevity*, *2019*.
[42] Ngonda, Frank. "In-vitro Anti-oxidant Activity and Free Radical Scavenging Potential of roots of Malawian Trichodesma zeylanicumm (burm. f.)." *Asian Journal of Biomedical and Pharmaceutical Sciences* 3.20 (2013): 21.
[43] Niksic, H., Becic, F., Koric, E., Gusic, I., Omeragic, E., Muratovic, S., ... & Duric, K. (2021). Cytotoxicity screening of Thymus vulgaris L. essential oil in brine shrimp nauplii and cancer cell lines. *Scientific reports*, *11*(1), 1-9.
[44] Ogbonna, P.C., Princewill-Ogbonna, I.L., Nzegbule, E.C., Mpamah, I.C. and Nwachukwu, N.G., 2018. Levels of biologically active compounds and some essential metal in parts of African oil bean tree (Pentaclethra macrophylla). *Journal of Applied Sciences and Environmental Management*, *22*(8), pp.1315-1320.
[45] Olusola, A. O., et al. "Evaluation of The Antimicrobial Activity of Saponins-Rich Fraction of Zanthoxylum zanthoxyloides Leaf." *Journal of Medical and Biological Science Research* 6.3 (2020): 29-34.
[46] Perumpail, B.J., Li, A.A., John, N., Sallam, S., Shah, N.D., Kwong, W., Cholankeril, G., Kim, D. and Ahmed, A., 2018. The Role of Vitamin E in the Treatment of NAFLD. *Diseases*, *6*(4), p.86.
[47] Rajamani, K., Thirugnanasambandan, S.S., Natesan, C., Subramaniam, S., Thangavel, B. and Aravindan, N., 2021. Squalene deters drivers of RCC disease progression beyond VHL status. *Cell Biology and Toxicology*, *37*(4), pp.611-631.
[48] Sado Kamdem, S.L., Belletti, N., Tchoumbougnang, F., Essia-Ngang, J.J., Montanari, C., Tabanelli, G., Lanciotti, R. and Gardini, F., 2015. Effect of mild heat treatments on the antimicrobial activity of essential oils of Curcuma longa, Xylopia aethiopica, Zanthoxylum xanthoxyloides and Zanthoxylum leprieurii against Salmonella enteritidis. *Journal of Essential Oil Research*, *27*(1), pp.52-60.
[49] Saleem, H., Zengin, G., Ahmad, I., Lee, J.T.B., Htar, T.T., Mahomoodally, F.M., Naidu, R. and Ahemad, N., 2019. Multidirectional insights into the biochemical and toxicological properties of Bougainvillea glabra (Choisy.) aerial parts: A functional approach for bioactive compounds. *Journal of pharmaceutical and biomedical analysis*, *170*, pp.132-138.
[50] Salma, S., Baig, S.G., ul Hasan, M.M., Ahmed, S. and Fatima, S.A., 2018. Analgesic and anti-inflammatory effects of Phaseolus vulgaris L. fixed oil in rodents. *Journal of Basic and Applied Sciences*, *14*, pp.174-179.
[51] Sarah, Q.S., Anny, F.C. and Misbahuddin, M., 2017. Brine shrimp lethality assay. *Bangladesh J Pharmacol*, *12*(2), p.5.
[52] Senguttuvan, J., Paulsamy, S., & Karthika, K. (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. *Asian Pacific journal of tropical biomedicine*, *4*, S359-S367.
[53] Shamran, D.J. and Abed, E.H., 2020. Plants-derived materials and their effects as anticancer agents: a review. *Annals of Tropical Medicine and Public Health*, *23*, pp.77-86.
[54] Shu, H., Chen, H., Wang, X., Hu, Y., Yun, Y., Zhong, Q., Chen, W. and Chen, W., 2019. Antimicrobial activity and proposed action mechanism of 3-carene against Brochothrix thermosphacta and Pseudomonas fluorescens. *Molecules*, *24*(18), p.3246.
[55] Sofowora, A. (1993). Screening plants for bioactive agents. *Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria*, 134-156.
[56] Tanase, C., Coșarcă, S. and Muntean, D.L., 2019. A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. *Molecules*, *24*(6), p.1182.

[57] Vieira, A.J., Beserra, F.P., Souza, M.C., Totti, B.M. and Rozza, A.L., 2018. Limonene: Aroma of innovation in health and disease. *Chemico-Biological Interactions*, *283*, pp.97-106.

[58] Vydrina, V.A., Kravchenko, A.A., Yakovleva, M.P., Ishmuratova, N.M. and Ishmuratov, G.Y., 2018. Stereoselective Synthesis of the Antileukemic Sesquiterpene (+)-Caparratriene from L-menthol and Tiglic Aldehyde. *Chemistry of Natural Compounds*, *54*(3), pp.461-463.

[59] Yakubogullari, N., Coven, F. O., Cebi, N., Coven, F., Coven, N., Genc, R., ... & Nalbantsoy, A. (2021). Evaluation of adjuvant activity of Astragaloside VII and its combination with different immunostimulating agents in Newcastle Disease vaccine. *Biologicals*, *70*, 28-37.

[60] Yang, H., Tian, T., Wu, D., Guo, D. and Lu, J., 2019. Prevention and treatment effects of edible berries for three deadly diseases: cardiovascular disease, cancer and diabetes. *Critical reviews in food science and nutrition*, *59*(12), pp.1903-1912.

[61] Yao, Y., Ding, D., Shao, H., Peng, Q. and Huang, Y., 2017. Antibacterial activity and physical properties of fish gelatin-chitosan edible films supplemented with D-Limonene. *International Journal of Polymer Science*, *2017*.