O.C.Ogundare et al /J. Pharm. Sci. & Res. Vol. 17(3), 2025, 67-74

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Antioxidant and Cytotoxic Effects of *Zanthoxylum zanthozyloides* Shoot Extracts: Potential for Anticancer and Attenuation of COVID-19 Outcomes

O.C. Ogundare[1], S.M. Akoro[1], A.O. Adepoju[1], M.A. Omotayo[1], S.A. Ogundare[2], I.V. Oludare[3], Abimbola A. Obayomi[1] and Oluwasegun M. Adekunle[1]

[1]*Lagos State University of Science and Technology, Ikorodu, Lagos State, Nigeria*
[2]*Ademola Hospital, Ijanikin, Lagos State, Nigeria*
[3]*University of Ilorin, Kwara State, Nigeria*

ogundareoyinlade@yahoo.com

Abstract

Reducing cancer risk and enhancing treatments and antioxidant defenses may help to reduce severe COVID-19 outcomes, especially among high-risk populations. Also, most cytotoxic plants are known for biological activities, particularly anticancer properties. Thus, this study investigates the secondary metabolites, antioxidant activity, and cytotoxicity of ethanol and saponin extracts derived from *Zanthozylum zanthozyloides* shoots. The plant ethanol shoot extract was screened for chemical components using gas chromatography-mass spectrometry (GC-MS). The saponin extract obtained from the ethanol extract was subjected to *in-vitro* antioxidant activity via 2, 2-Diphenyl-1- picrylhydrazyl (DPPH), nitric oxide (NO), and hydrogen peroxide (H_2O_2) radical scavenging properties in comparison to ascorbic acid. The plant cytotoxic potential was evaluated using brine shrimp lethality (BSL) assay and compared to doxorubicin. The chemical analysis revealed the presence of bioactive components such as D-limonene, caparratriene, chloroacetic acid, dodec-9-ynyl ester, and vitamin E. Antioxidant assays demonstrated that the ethanol extract exhibited significant ($p<0.05$) DPPH scavenging activity (33.42 μg/mL), outsurpassing the saponin extract (42.88 μg/mL) but comparable to ascorbic acid (39.32 μg/mL). Similar results were observed in NO (32.44 μg/mL) and hydrogen peroxide scavenging assays (39.91 μg/mL). Furthermore, the extracts showed a notable cytotoxic potential in the BSL assay. The saponin extract (LC_{50} of 78.69 μg/mL) and the ethanol extract (LC_{50} of 88.25 μg/mL) were significantly ($p<0.05$) less potent than doxorubicin ($LC5_{50}$ of 8.62 μg/mL). These findings indicate that *Z. zanthozyloides* possesses bioactive compounds with antioxidant and cytotoxic properties that could reduce the severity of COVID-19 in cancer-prone individuals and frontline medical personnel.

Keywords: *Cancer, Secondary metabolites, Antioxidant, Cytotoxicity, COVID-19*

Introduction

The ongoing coronavirus disease-2019 (COVID-19) pandemic has highlighted the need for preventive strategies for microbial infections such as those resulting from viruses like the severe acute respiratory syndrome coronavirus (SARS-CoV)-2. This is of utmost importance in individuals with underlying conditions like cancer, which increases susceptibility to severe illness and mortality (Behl et al., 2021). Moreover, the SARS-CoV-2-caused disease is characterized by elevated oxidative stress, causing further tissue damage and exacerbating the body's inflammatory response. The current landscape of COVID-19 management encompasses various strategies, including antiviral medications, immunomodulatory drugs, and traditional therapies in addition to the use of vitamin and mineral supplements, underscoring the necessity for diverse treatment options to tackle the complexity of the disease (Al-kuraishy et al., 2022; Pascarella et al., 2020). Thus, by neutralizing harmful free radicals, antioxidants play a vital role in reducing oxidative stress-related damage, thus potentially mitigating severe COVID-19 symptoms (Omoruyi et al., 2021; Yaqinuddin et al., 2022). Conversely, cancer patients, who often have compromised immune systems, may benefit from antioxidant-rich compounds that can help bolster their defenses.

Interestingly, many traditionally used herbal medicines contain numerous bioactive compounds with antioxidants and anti-inflammatory and cytotoxic properties that target malignant cells. These constituents may inhibit cellular oxidation, scavenge free radicals, and induce apoptosis in cancer cells, making them valuable for cancer prevention and immune support in COVID-19 vulnerable individuals and health frontiers.

As global efforts continue to explore effective treatments for COVID-19, natural compounds from plants such as *Zanthoxylum zanthoxyloides*, having demonstrated antiproliferative activity and potential antiviral effects, is a promising avenue for further insights into antiviral strategies (Andima et al., 2019; Mawthoh et al., 2023). *Z. zanthozyloides*, commonly found in tropical regions, is a plant with reported medicinal properties. Ethnobotanical studies highlight its use in traditional African medicine for pain relief and antimicrobial and anticancer treatments (Okagu et al., 2021). Its secondary metabolites—including saponins, alkaloids, and terpenoids, are believed to contribute to these health benefits. These chemical components may act singly or synergize with other components to produce the desired pharmacological effects.

Saponins, a class of naturally occurring glycosides, are particularly notable for their wide range of biological activities, including antioxidant, anticancer, and anti-inflammatory effects (Elekofehinti et al., 2021; Goldson-Barnaby & Williams, 2016; Hu et al., 2012; Liu et al., 2022). They work by modulating cellular antioxidant pathways and promoting the immune system's natural defenses through anti-inflammatory and oxidative stress management and as effective adjuvants, enhancing the efficacy of vaccines in various animal models (Mengie et al., 2021; Wang et al., 2020). These properties may be crucial in preventing and possibly ameliorating COVID-19 symptoms. This study evaluated the *Z. Zanthozyloides*'*s* secondary metabolites, antioxidant activities, and cytotoxic effects, particularly its ethanol and saponin-rich extracts, to determine potential benefits in reducing COVID-19 severity among high-risk individuals.

METHODS

2.1 Collection of plant materials

Fresh matured shoots of *Z. zanthozyloides* were obtained from a forest in Oyo town, Oyo State, Nigeria, and a voucher specimen was submitted to the herbarium at the University of Lagos, Akoka, for verification (LUH 6909). The sample was air-dried at room temperature in the laboratory before being ground into a coarse powder and set aside for extraction.

2.2 Extraction of plant samples

The ground sample of shoots of *Z. zanthozyloides* (800g) was extracted in 95% ethanol in a ratio of 1:4 (w/v) by maceration for 72 hours with occasional stirring. The mixture was filtered, and a portion was subjected to phytochemical screening. At the same time, the remaining filtrate was evaporated to dryness in the glass flask of a rotary evaporator at 45°C. The greenish-brown concentrated extract regarded as ethanol extract of *Z. zanthozyloides* shoots (EEZZ) was kept in a refrigerator at -20°C for future use.

2.3 Phytochemical screening

The methods described by Harborne (1984), Rice-Evans et al. (1996), and Sofowora (1993) were adopted for the screening of phytochemicals in the shoot of *Z. zanthozyloides*.

2.4 Extraction of Saponins from *Z. zanthozyloides*

Saponins from *Z. zanthozyloides* shoots were isolated from the greenish-brown concentrated ethanol extract shoot of *Z. zanthozyloides* (EEZZ) above according to the combined procedures described by Olusola et al. (2020). In this case, the EEZZ was partitioned with hexane and water (1:2, v/v). The mixture was shaken thoroughly for 30 minutes on a shaker and then left at room temperature overnight. The water layer was concentrated and partitioned into ethyl acetate and n-butanol (1:3, v/v). The butanol fraction was concentrated to obtain crude saponin extract, which was designated SEZS.

2.5 Antioxidant activity of shoot extract of *Z. zanthozyloides*

A stock solution (1.0 mg/mL) of saponin extract of *Z. zanthozyloides* shoot (SEZS) was prepared in ethanol and used to determine the antioxidant activities. The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO), and hydrogen peroxide (H_2O) radical scavenging activities of SEZS were determined using standard methods (Alam et al., 2013; Muthal et al., 2015; Nanyonga et al., 2013)). The experiment was performed in triplicates, and the scavenging free radical was estimated using the equation below. The results were compared to ascorbic acid.

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

In evaluating the DPPH scavenging activity, a 0.1 mM DPPH solution was prepared in ethanol. From this solution, 1.0 mL was added to tubes containing serially diluted SEZS solutions in ethanol at concentrations ranging from 10 to 100 μg/mL. The test solutions were incubated in a dark drawer at 37°C for 30 minutes. The absorbance of the solutions was measured at 517 nm, using ethanol as the blank. Ascorbic acid was used as a control in the study. A reduced absorbance of the reaction mixture indicates enhanced free radical scavenging activity. The DPPH radical scavenging activity of SEZS was calculated using the above-mentioned equation. The antioxidant activity of SEZS was expressed as IC_{50}, defined as the concentration of SEZS in μg/mL required to inhibit the formation of DPPH radicals by 50%.

The scavenging of nitric oxide (NO) generated from sodium nitroprusside by the SEZS was assessed using the method outlined by Alam *et al*. (2013). To prepare the Griess reagent (0.33%), 1.0 mL of sulfanilic acid reagent (0.33%) was combined with 20% glacial acetic acid. This solution was allowed to react with an equal volume of naphthyl ethylenediamine dichloride (0.1% w/v) for 5 minutes at room temperature.

Subsequently, 2 mL of 10 mM sodium nitroprusside (prepared in 0.5 mL phosphate-buffered saline at pH 7.4) was mixed with 0.5 mL of SEZS at various concentrations ranging from 10 μg/mL to 100 μg/mL. The resulting mixture was then incubated at 25°C for 3 hours. Following incubation, 0.5 mL of the solution was withdrawn and combined with 0.5 mL of Griess reagent, allowing it to react for 30 minutes. The absorbance of the final solution was read at 546 nm, and the extent of NO radicals inhibition was determined according to the equation below.

$$\%\, inhibition\ of\ NO\ radical = \frac{(absorbance\ before\ the\ reaction - absorbance\ after\ the\ reaction)100}{absorbance\ before\ reaction}$$

The ability of *Z. zanthozyloides* shoot extracts to neutralize hydrogen peroxide radicals was also determined. The hydrogen peroxide solution (40 Mm) was prepared in a pH 7.4 phosphate buffer. This preparation was designated as the control, and its absorbance at 560nm was determined using a UV spectrophotometer in contradiction of a blank solution containing phosphate buffer without hydrogen peroxide. For the sample (SEZS), 0.1mg/mL of the extract was with the hydrogen peroxide solution, and absorbance was measured at 560nm using a UV spectrophotometer against the blank solution. The amount of hydrogen peroxide scavenged by the extract and the standard compound was calculated using the equation below.

$$\%\, Scavenging\ \text{hydrogen peroxide radicals}\ activity = \frac{(absorbance\ of\ control - absorbance\ of\ sample)100}{absorbance\ of\ control}$$

2.6 Brine shrimp lethality (BSL) assay of extracts of *Z. zanthozyloides*

The protocol previously described by Ogundare et al. (2023) was adopted. The cytotoxicity of the extract (SEZS) was assessed using the brine shrimp lethality assay (BSLA). A sea salt solution (38 g/L) was prepared, adjusted to pH 8.5, filtered, and used to hatch brine shrimp eggs obtained from a pet shop in Ikeja, Nigeria, in a controlled environment with constant light and aeration. Stock solutions of the extract (concentrations from 10 μg/mL to 1000 μg/mL) were prepared in 0.1% DMSO alongside a control solution of 0.1% DMSO. Both the extract solutions and DMSO were sterilized. After 24 hours, ten nauplii were transferred to test tubes with seawater, and 2.5 mL of the extract was added. The mixture was made to 10mL with seawater. After 48 hours, the nauplii's survival was monitored based on their ability to undergo a forward movement. The number of active nauplii was counted, and the percentage of mortality was recorded. The procedure was carried out in triplicates.

2.7 Statistical analysis

This statistical analysis was conducted using GraphPad Prism software. One-way analysis of variance (ANOVA) based on Dunnett's post hoc test was utilized to analyze and gather all analytical data, graphs, and significant differences between treatments using GraphPad Prism software.

RESULTS AND DISCUSSION

Investigating biologically active substances in *Z. zanthoxyloides* shoots provides indispensable insights into its potential role in ameliorating COVID-19 outcomes, particularly in cancer patients. In this study, the analysis of ethanol extract of *Z. zanthozyloides* shoots revealed the presence of bioactive compounds such as saponins, flavonoids, tannins, phenolics, and glycosides (Table 1), which are associated with antibacterial, antioxidant, anti-inflammatory, and anticancer effects (Ali et al., 2021; Olusola et al., 2020; Olusola et al., 2021). Notably, saponins, a key component of the plant, have been linked to its medicinal properties. The saponin extract yielded 6.03% (w/w), which was slightly lower than the 8.2% obtained through methanol extraction in previous research (Olusola et al., 2020a). This variation highlights the influence of solvents and methods on the extraction efficiency of bioactive compounds.

Further analysis identified a number of bioactive compounds components (Figure 1). These components include D-limonene, squalene, 3-carene, caparratriene, chloroacetic acid, dodec-9-ynyl ester, and vitamin E, all contributing to the plant's biological activities (Table 2). D-limonene is noted for its antioxidant, anticancer, and antiviral properties (Anandakumar et al., 2021; Araújo-Filho et al., 2021; Costa et al., 2019; Shu et al., 2019). Additionally, 3-carene, a monoterpene commonly found in many plants, possesses antimicrobial and antifungal effects (Kang et al., 2019; Shu et al., 2019). Caparratriene is a well-known cytotoxic and antileukemic agent (Ramya, 2022; Vydrina et al., 2018). Squalene, a natural lipophilic biomolecule of the class triterpenes, is known for its antioxidant, immunostimulant, and anticancer activities (Micera et al., 2020; Rajamani et al., 2020; Yakubogullari et al., 2021). Chloroacetic acid, dodec-9-ynyl ester possess anti-inflammatory and neuromuscular protective properties and present a promising avenue for therapeutic intervention in cancer and COVID-19, particularly in managing inflammation and preserving neuromuscular function (Diorio et al., 2020; Mohammad et al., 2019). Vitamin E, widely recognized for its anticancer, anti-inflammatory, and antioxidant properties, is also considered a potential adjunct in COVID-19 treatment (Abraham et al., 2018; Salma et al., 2018; Tavakol & Seifalian, 2022). These compounds, particularly D-limonene, caparratriene, chloroacetic acid, dodec-9-ynyl ester and vitamin E collectively contribute to the anticancer, antiviral, anti-inflammatory, and neuromuscular protective, antioxidant, and cytotoxic properties of *Z. zanthoxyloides*, making extracts from *Z. zanthoxyloides* potentially therapeutic for managing oxidative stress in cancer and viral infections.

Interestingly, the results from the antioxidant analyses indicate that the *Z. zanthozyloides* extracts exhibit a significant antioxidant potential, characterized by a dose-dependent response (Figure 2). Notably, the ethanol extract showed a comparable antioxidant to the widely recognized antioxidant ascorbic acid (Figure 3). Specifically, the ethanol extract demonstrated a DPPH radical scavenging capacity of 33.42 μg/mL, which was significantly higher than that of the saponin extract at 42.88 μg/mL and comparable to ascorbic acid at 39.32 μg/mL. A reduced IC_{50} value indicates an increased level of antioxidant activity. This suggests that *Z. zanthozyloides* could serve as a valuable source of natural antioxidants with therapeutic potential for cancer treatment and mitigating oxidative stress-related conditions, including COVID-19, aligning with previous studies that have highlighted the antioxidant potential of various *Zanthoxylum* species (Ayoka et al., 2022; Ogundare et al., 2022; Olusola et al., 2020). Moreover, the ethanol extract's DPPH radical scavenging capacity further suggests a high concentration of phenolic and flavonoid compounds in the *Z. zanthozyloides* shoot extracts. These compounds are known for their potent radical scavenging properties.

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

Phytochemicals	Phenolics	Flavonoids	Alkaloids	Saponins	Tannins	Glyco sides	Steroids	Carbohydrates
Ethanol extract	+	+	+	+	+	+	+	+

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

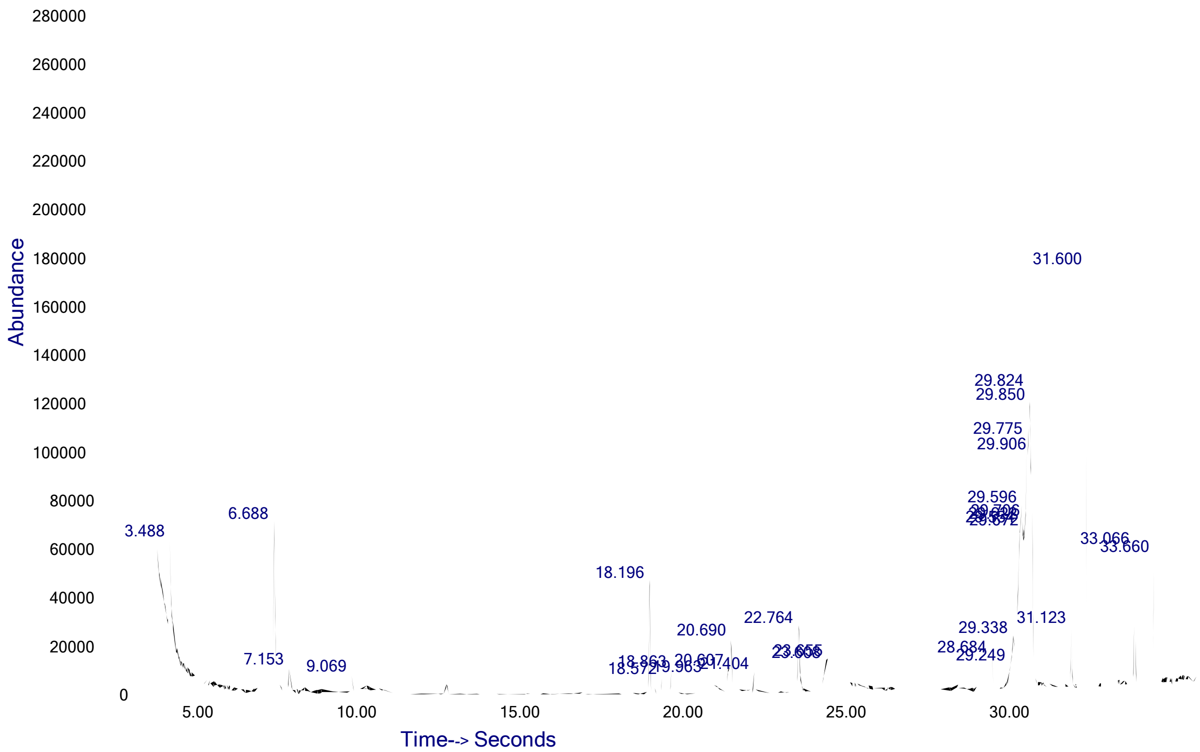

Figure 1: GC-MS analysis of ethanol extract of *Z. zanthozyloides* shoot

Table 2: Some bioactive components of ethanol extract of *Z. zanthozyloides* shoots using GC-MS analysis

Retention time	Similarity index	Area (%)	Compound	Molecular mass	Biological activities	References
6.686	98	6.65	D-Limonene	136.238	Anticancer, antitumor, antibacterial, antioxidant, anti-inflammatory, antidiabetic, gastroprotective and antiviral.	(Anandakumar et al., 2021; Araújo-Filho et al., 2021; Costa et al., 2019)
7.155	47	0.72	3-Carene	136.24	Antifungal and antimicrobial.	(Kang et al., 2019; Shu et al., 2019)
29.83	70	8.52	Caparratriene	206.4	Cytotoxic and antileukemia.	(Ramya, 2022; Vydrina et al., 2018)
29.85	80	7.88	Chloroacetic acid, dodec-9-ynyl ester	262.8	Anti-inflammatory and neuromuscular protection.	(Diorio et al., 2020; Mohammad et al., 2019)
31.124	78	1.52	Squalene	410.73	Antibacterial, Antioxidant, Antitumor, Cancer-Preventive, Chemopreventive, Immunostimulant.	(Micera et al., 2020; Rajamani et al., 2020; Yakubogullari et al., 2021)
33.659	94	3.52	Vitamin E	430.71	Analgesic, anti-inflammatory, antioxidant, antitumor, and antileukemic.	(Abraham et al., 2018; Salma et al., 2018; Tavakol & Seifalian, 2022)

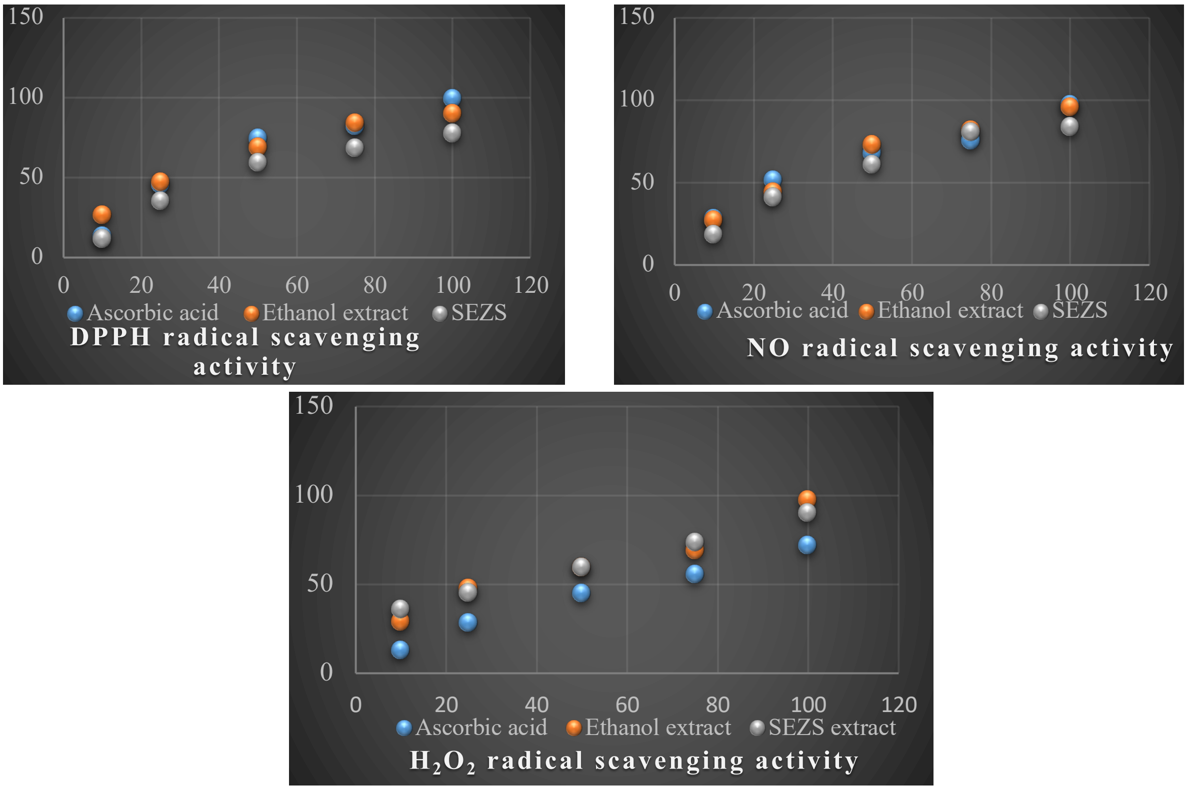

Figure 2: Inhibition of free radicals by *Z. zanthozyloides* shoots

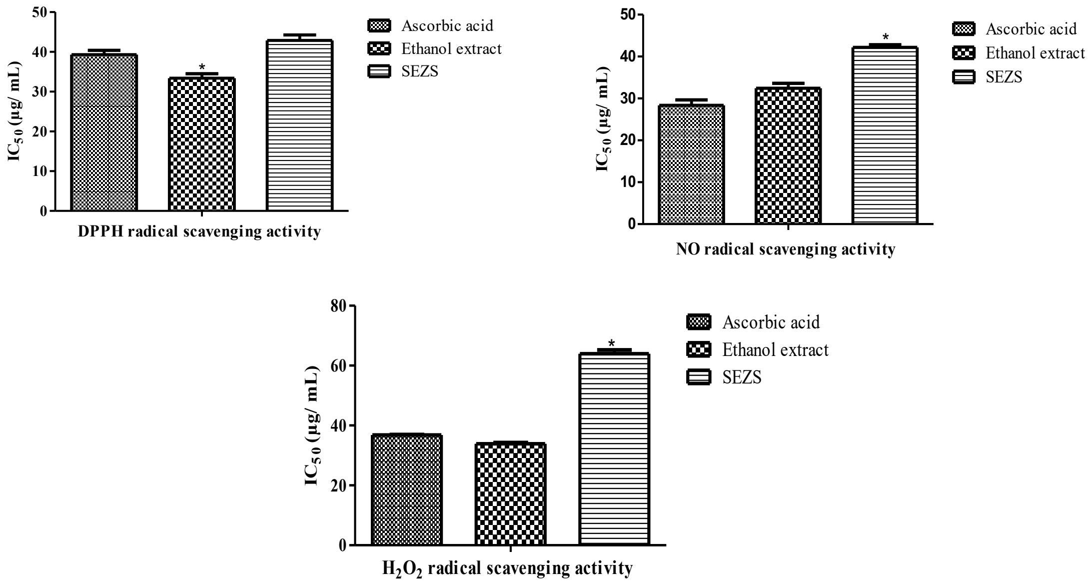

Figure 3: Free radical scavenging activities of *Z. zanthozyloides* shoots
Data are represented in terms of mean ± SEM of triplicate readings. The (*) implies a significant difference at (*p<0.05*) when compared with ascorbic acid.

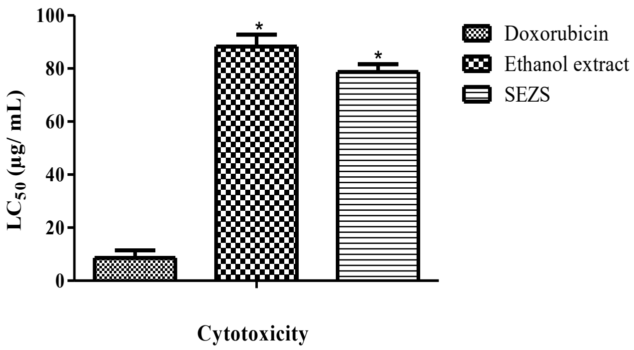

Figure 4: Cytotoxic potential of *Z. zanthozyloides* shoots
Data are represented in terms of mean ± SEM of triplicate readings. The (*) implies a significant difference at ($p<0.05$) when compared with doxorubicin.

In addition to the DPPH assay, the nitric oxide (NO) scavenging assay further corroborated the antioxidant efficacy of the ethanol extract, with significant differences observed among the extracts. The ethanol extract's ability to scavenge NO radicals at 32.44 µg/mL was again superior to the saponin extract (42.18 µg/mL), reinforcing that ethanol extracts may be more effective in neutralizing free radicals. NO radicals are key mediators in oxidative stress and inflammation. Thus, the result supports the extract's potential for addressing inflammatory conditions such as those observed in severe COVID-19 cases (Rahban et al., 2020; Valaei et al., 2022). Similarly, in the H_2O_2 scavenging assay, the lower scavenging capacity of the saponin extract for hydrogen peroxide (H_2O_2) at 63.98 µg/mL compared to the ethanol extract and ascorbic acid indicates a potential limitation of saponins in antioxidant applications. These findings corroborate earlier research that identifies polyphenols and flavonoids as significant contributors to the antioxidant activity observed in plant-based extracts (Zhang & Tsao, 2016). Moreover, the results are in agreement with other studies that have reported varying antioxidant activities among different extracts from the *Zanthoxylum* species. This variation underscores the critical role that extraction methodologies and solvent selection play in influencing the antioxidant potential of these extracts (Lelyana et al., 2021).

The brine shrimp lethality assay revealed notable cytotoxic activity for the saponin extract with an LC_{50} of 78.69 µg/mL, outperforming the ethanol extract (88.25 µg/mL) but remaining less potent than the standard anticancer drug doxorubicin (8.62 µg/mL), a well-known chemotherapeutic agent (Figure 4). Saponins are known for their cytotoxic and antiviral properties, often involving membrane disruption and apoptosis induction in cancer cells (Elekofehinti et al., 2021; Falade et al., 2021). In contrast, the ethanol extract's relatively lower cytotoxicity may stem from the presence of a broader spectrum of compounds, some of which might counterbalance its cytotoxic effects. This divergence between the extract's antioxidant and cytotoxic activities is particularly significant, as it suggests that the ethanol extract may be more suitable for conditions requiring oxidative stress mitigation without causing significant cytotoxicity. Conversely, the saponin extract could be explored further for its potential as an anticancer agent. Notably, the cytotoxic potential of the extracts of *Z. zanthoxyloides* shoots is in line with previous research that has documented the cytotoxic properties of *Zanthoxylum* species against various cancer cell lines, suggesting that specific bioactive compounds such as saponins and flavonoids present in these plants may have therapeutic potential in cancer treatment (Alam et al., 2017; Ashraf et al., 2013; Elekofehinti et al., 2021).

Also, the diverse secondary metabolites, including alkaloids and flavonoids, detected in *Z. zanthoxyloides* shoot's extracts are probably responsible for the observed cytotoxicity and antioxidant activities, presenting promising therapeutic applications. The ethanol extract's strong radical scavenging ability positions it as a candidate for managing oxidative stress-related disorders, including the excessive inflammation associated with cancer and severe COVID-19 cases (Omoruyi et al., 2021; Seneviratne et al., 2022; Yaqinuddin et al., 2022). Meanwhile, the cytotoxic activity of the saponin extract in healthy nauplii could be harnessed in anticancer therapies, especially if future studies confirm its selectivity for cancer cells over normal cells. It may be offered as a natural supplement to existing chemotherapies.

CONCLUSION

In conclusion, the antioxidant and cytotoxic properties of *Z. zanthozyloides* extracts underscore the potential of this plant as a valuable resource in the search for natural compounds with antiradical and cytotoxicity and a possible anticancer property that could help patients and frontline medical personnel reduce the severity of COVID-19 outcomes. Future research is focused on isolating specific active components, elucidating their mechanisms of action, and exploring their efficacy in combination with conventional therapies to maximize their therapeutic potential.

Acknowledgment
The authors of this manuscript would like to express their gratitude to the management of our institution for their support during this research.

Conflict Of Interest

Regarding this paper, there is no conflict of interest to declare.

References

Abraham, A., Kattoor, A. J., Saldeen, T., & Mehta, J. L. (2018). Vitamin E and its anticancer effects. *In Critical Reviews in Food Science and Nutrition,* 59, (17), 2831–2838.

Alam, F., Najum us Saqib, Q., & Waheed, A. (2017). Cytotoxic activity of extracts and crude saponins from *Z. armatum* DC. against human breast (MCF-7, MDA-MB-468) and colorectal (Caco-2) cancer cell lines. *BMC Complementary and Alternative Medicine,* 17(1). https://doi.org/10.1186/s12906-017-1882-1

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and *in-vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal,* 21(2), 143–152.

Ali, M. N., Ndahi, J., Abdullahi, A., & Yelwa, J. (2021). Phytochemicals, chemical composition and antioxidant profile of the crude extracts and essential oil of Mitracarpus scaber (Goga masu). *Journal of Research in Chemistry,* 2(2), 27–31.

Al-kuraishy, H. M., Al-Fakhrany, O. M., Elekhnawy, E., Al-Gareeb, A. I., Alorabi, M., De Waard, M., Albogami, S. M., & Batiha, G. E. S. (2022). Traditional herbs against COVID-19: back to old weapons to combat the new pandemic. *European Journal of Medical Research,* 27(1), 1–11. https://doi.org/10.1186/s40001-022-00818-5

Anandakumar, P., Kamaraj, S., & Vanitha, M. K. (2021). D-limonene: A multifunctional compound with potent therapeutic effects. *Journal of Food Biochemistry,* 45(1). Blackwell Publishing Ltd. https://doi.org/10.1111/jfbc.13566

Andima, M., Coghi, P., Yang, L. J., Wai Wong, V. K., Mutuku Ngule, C., Heydenreich, M., Ndakala, A. J., Yenesew, A., & Derese, S. (2019). antiproliferative activity of secondary metabolites from *Z. zanthoxyloides* Lam: *In-vitro* and *in-silico* Studies. *Pharmacognosy Communications,* 10(1), 44–51. https://doi.org/10.5530/pc.2020.1.8

Araújo-Filho, H. G. de, dos Santos, J. F., Carvalho, M. T. B., Picot, L., Fruitier-Arnaudin, I., Groult, H., Quintans-Júnior, L. J., & Quintans, J. S. S. (2021). Anticancer activity of limonene: A systematic review of target signaling pathways. *In Phytotherapy Research* (Vol. 35, Issue 9, pp. 4957–4970). John Wiley and Sons Ltd.

Ashraf, M. F., Aziz, M. A., Stanslas, J., Ismail, I., & Kadir, M. A. (2013). Assessment of antioxidant and cytotoxicity activities of saponin and crude extracts of *Chlorophytum borivilianum*. 2013.

Ayoka, T. O., Nwachukwu, N., Ene, A. C., Igwe, C. U., Ogara, A. L., & Nnadi, C. O. (2022). *In-vitro* antioxidant activity and acute toxicity of the alkaloidal constituents of *Z. zanthoxyloides* leaves. *Tropical Journal of Natural Product Research,* 6(2), 276–280.

Costa, M. D. S., Rocha, J. E., Campina, F. F., Silva, A. R. P., Da Cruz, R. P., Pereira, R. L. S., Quintans-Júnior, L. J., De Menezes, I. R. A., Adriano, A. A., De Freitas, T. S., Teixeira, A. M. R., & Coutinho, H. D. 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, 158–161.

Diorio, C., Henrickson, S. E., Vella, L. A., McNerney, K. O., Chase, J., Burudpakdee, C., Lee, J. H., Jasen, C., Balamuth, F., Barrett, D. M., Banwell, B. L., Bernt, K. M., Blatz, A. M., Chiotos, K., Fisher, B. T., Fitzgerald, J. C., Gerber, J. S., Gollomp, K., Gray, C., … Bassiri, H. (2020). Multisystem inflammatory syndrome in children and COVID-19 are distinct presentations of SARS–CoV-2. *Journal of Clinical Investigation,* 130(11), 5967–5975. https://doi.org/10.1172/JCI140970

Elekofehinti, O. O., Iwaloye, O., Olawale, F., & Ariyo, E. O. (2021). Saponins in cancer treatment: Current progress and future prospects. *Pathophysiology,* 28(2), 250–272.

Falade, V. A., Adelusi, T. I., Adedotun, I. O., Abdul-Hammed, M., Lawal, T. A., & Agboluaje, S. A. (2021). *In-silico* investigation of saponins and tannins as potential inhibitors of SARS-CoV-2 main protease (Mpro). *In-silico Pharmacology*, 9(1).

Harborne, J. B. (1984). Phytochemical Methods (2nd ed.). Springer Netherlands. https://doi.org/10.1007/978-94-009-5570-7

Kang, G. Q., Duan, W. G., Lin, G. S., Yu, Y. P., Wang, X. Y., & 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).

Lelyana, S., Widyarman, A. S., & Amtha, R. (2021). Indonesian andaliman fruit (*Z. acanthopodium* DC.) extract suppresses the expression of inflammatory mediators on fibroblasts cells *in-vitro*. Pharmaceutical Sciences Asia, 48(5), 491–497.

Mawthoh, A. B., Seram, D., Singh, K. A., & Watt, H. J. (2023). Applications of Prickly Ash (Zanthoxylum spp.): Potential in traditional science, modern science, and agriculture. *Journal of Food Chemistry and Nanotechnology,* 9(1), S117–S131. United Scientific Group. https://doi.org/10.17756/jfcn.2023-s1-016

Micera, M., Botto, A., Geddo, F., Antoniotti, S., Bertea, C. M., Levi, R., Gallo, M. P., & Querio, G. (2020). Squalene: More than a step toward sterols. *Antioxidants*, 9(8), 1–14.

Mohammad, H., Prabhu, K., Rao, M. R. K., Sundram, L., Dinakar, S., Sathish Kumar, M., & Vijayalakshmi, N. (2019). The gas chromatography-mass spectrometry study of one ayurvedic pain relieving oil "*Mahamasha Thailam*." *Drug Invention Today,* 12(7), 1524–1527.

Muthal, A. P., Rojatkar, S. R., & Bodhankar, S. L. (2015). *In-vitro* free radicals scavenging and antioxidant activity of rice bran extract. *Pharmacologia,* 6(8), 377–385.

Nanyonga, S. K., Opoku, A. R., Lewu, F. B., Oyedeji, O. O., Singh, M., & Oyedeji, A. O. (2013). Antioxidant activity and cytotoxicity of the leaf and bark extracts of *Tarchonanthus camphorates*. *Tropical Journal of Pharmaceutical Research,* 12(3), 377–383.

Ogundare, O. C., Adedosu, O. T., Afolabi, O. K., Adeleke, G. E., Akinboro, T., Daniel, A. A., Akoro, S. M., & Oludare, V. I. (2023). Ethnobotanical survey, physiochemical composition and preliminary cytotoxic evaluation of some medicinal plants with anticancer potential from certain areas in South-West Nigeria. *Annual Research & Review in Biology,* 38(1), 27–42.

Ogundare, O. C., Akoro, S. M., Adepoju, A. O., Oshin, T. T., Sanyaolu, V. I., Ogundare, S. A., Oluremilekun, O., Oludare, V. I., & Bukola, K. T. (2022). Bioactive, antioxidant and gas chromatography-mass spectrometer analyses of *Z. zanthoxyloides.* 8(9), 1–9.

Olusola, A. O., Elekan, A. O., Olusola, A. O., Ogidan, T. O., & Ekun, O. E. (2020). Evaluation of *in-vitro* antioxidant activity of saponin-rich fraction from leaves *of Z. zanthoxyloides. Environmental and Experimental Biology*, 18(3), 175–181.

Olusola, A. O., Elekan, A. O., Olusola, A. O., Ogidan, T. O., Ekun, O. E., & Onoagbe, I. O. (2020b). Evaluation of the antimicrobial activity of saponins-rich fraction of Z.

zanthoxyloides leaf. *Journal of Medical and Biological Science Research,* 6(3), 25–28.

Olusola, A. O., Ogidan, T. O., Olusola, A. O., Elekan, A. O., Ekun, O. E., & Onoagbe, I. O. (2021). Antimicrobial and antioxidant properties of flavonoids-rich fraction of *Z. zanthoxyloides* leaf. *Journal of Bio Innovation,* 10(1), 359–377.

Omoruyi, B. E., Ighodaro, D. I., & Lewu, F. B. (2021). Analysis of some natural products for prevention of diseases associated with COVID-19. *African Journal of Biomedical Research,* 24(1), 11–23.

Pascarella, G., Strumia, A., Piliego, C., Bruno, F., Del Buono, R., Costa, F., Scarlata, S., & Agrò, F. E. (2020). COVID-19 diagnosis and management: a comprehensive review. *Journal of Internal Medicine,* 288(2), 192–206. https://doi.org/10.1111/joim.13091

Rahban, M., Habibi-Rezaei, M., Mazaheri, M., Saso, L., & Moosavi-Movahedi, A. A. (2020). Antiviral potential and modulation of nrf2 by curcumin: Pharmacological implications. *Antioxidants,* 9(12), 1–24. https://doi.org/10.3390/antiox9121228

Rajamani, K., Thirugnanasambandan, S. S., Natesan, C., Subramaniam, S., Thangavel, B., & Aravindan, N. (2020). Squalene deters drivers of RCC disease progression beyond VHL status. *Cell Biology and Toxicology.* https://doi.org/10.1007/s10565-020-09566-w

Ramya, R. (2022). GC-MS Analysis of Bioactive Compounds in Ethanolic Leaf Extract of *Hellenia speciosa* (J.Koenig) S.R. Dutta. *Applied Biochemistry and Biotechnology,* 194(1), 176–186.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine,* 20(7), 933–956.

Salma, Sadia Ghousia Baig, Muhammad Mohtasheem ul Hasan, Salman Ahmed, & Syeda Anum Fatima. (2018). Analgesic and anti-inflammatory effects of *Phaseolus vulgaris* L. fixed oil in rodents. *Journal of Basic & Applied Sciences,* 14, 174–179.

Seneviratne, S. L., Wijerathne, W., Yasawardene, P., & Somawardana, B. (2022). COVID-19 in cancer patients. *Transactions of the Royal Society of Tropical Medicine and Hygiene* (Vol. 116, Issue 9). https://doi.org/10.1093/trstmh/trac015

Shu, H., Chen, H., Wang, X., Hu, Y., Yun, Y., Zhong, Q., Chen, W., & Chen, W. (2019). Antimicrobial activity and proposed action mechanism of 3-Carene against *Brochothrix thermosphacta* and *Pseudomonas fluorescens*. *Molecules,* 24(18).

Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Limited. Ibadan, Nigeria.

Tavakol, S., & Seifalian, A. M. (2022). Vitamin E at a high dose as an anti-ferroptosis drug and not just a supplement for COVID-19 treatment. *In Biotechnology and Applied Biochemistry* (Vol. 69, Issue 3, pp. 1058–1060). John Wiley and Sons Inc.

Valaei, A., Azadeh, F., Mostafavi Niaki, S. T., Salehi, A., Shakib Khoob, M., Mirebrahimi, S. H. O., Kazemi, S., & Hosseini, S. M. (2022). Antioxidant and anticancer potentials of the olive and sesame mixture against dimethylhydrazine-induced colorectal cancer in Wistar Rats. *BioMed Research International,* 2022, 5440773.

Vydrina, V. A., Kravchenko, A. A., Yakovleva, M. P., Ishmuratova, N. M., & Ishmuratov, G. Y. (2018). Stereoselective synthesis of the antileukemic sesquiterpene (+)-caparratriene from L-menthol and Tiglic aldehyde. *Chemistry of Natural Compounds,* 54(3), 461–463.

Yakubogullari, N., Coven, F. O., Cebi, N., Coven, F., Coven, N., Genc, R., Bedir, E., & Nalbantsoy, A. (2021). Evaluation of adjuvant activity of Astragaloside VII and its combination with different immunostimulating agents in Newcastle Disease vaccine. *Biologicals,* 70(01), 28–37.

Yaqinuddin, A., Ambia, A. R., & Alaujan, R. A. (2022). Immunomodulatory Effects of vitamin D and vitamin C to improve immunity in COVID-19 patients. *Journal of Health and Allied Sciences NU,* 12(01), 1–6. https://doi.org/10.1055/s-0041-1730084

Zhang, H., & Tsao, R. (2016). Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science,* 8, 33–42.