ISRG Journal of Multidisciplinary Studies (ISRGJMS)

ISRG PUBLISHERS
Abbreviated Key Title: isrg j. multidiscip. Stud.
ISSN: 2584-0452 (Online)
Journal homepage: https://isrgpublishers.com/isrgjms/
Volume – II Issue - VII (July) 2024
Frequency: Monthly

Phytochemicals Profile, Antioxidant and Antibacterial Activity of Detarium Microcarpum Against Gastrointestinal Bacteria

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| **Received:** 09.07.2024 | **Accepted:** 13.07.2024 | **Published:** 14.07.2024

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Abstract

Detarium microcarpum is used by different ethnic groups for the treatment of various diseases in Nigeria and several parts of West Africa. The aim was to assess the antioxidant and antibacterial properties of both N-hexane and chloroform extracts of D. microcarpum stem bark against S. aureus, and P. aeruginosa. The two clinical isolates were collected from the Central Laboratory of Modibbo University Yola and were reconfirmed using culture, microscopy, and some biochemical tests. The antibacterial activity of the stembark extracts against the isolates was tested using the agar well diffusion method. The phytochemical assay revealed the presence of alkaloids, saponins, steroids, Phenols, and flavonoids in both fractions. The plant extracts exhibited antibacterial potential against the tested organisms at different concentrations (100mg/mL, 50mg/mL, 25mg/mL, and 12.5mg/mL). D. microcarpum stem bark extracts, particularly the N-hexane and chloroform fractions, exhibited significant antibacterial activity against clinical isolates of Pseudomonas aeruginosa (21.17 ± 0.73 mm to 6.53 ± 0.19 mm and 19.63 ± 0.47 mm to 5.30 ± 0.30 mm, respectively) and Staphylococcus aureus (17.90 ± 0.27 mm to 5.10 ± 0.21 mm and 14.07 ± 0.22 mm to 4.33 ± 0.24 mm, respectively). These extracts displayed varied inhibitory effects, with Ciprofloxacin serving as a control with consistent 40 mm inhibition zones for both bacteria. FTIR analysis of the N-hexane extract revealed peaks indicative of functional groups such as O-H stretching (3253.91 cm-1), N-H stretching (2921.51 cm-1), imine/oxime (1692.15 cm-1), and C=C conjugated alkenes (1605.73 cm-1). Similarly, the chloroform extract exhibited peaks corresponding to O-H stretching (3253.32 cm-1), N-H stretching (2924.10 cm-1), imine/oxime (1692.93 cm-1), and C=C conjugated alkenes (1606.15 cm-1), along with additional peaks in the fingerprint region. The total antioxidant capacity assays showed that the chloroform fraction had lower antioxidant capacity

DOI: 10.5281/zenodo.12740667

(32.93 ± 0.64 µg/mL AAE) compared to the N-hexane fraction (54.74 ± 1.079 µg/mL AAE). However, the chloroform extract demonstrated higher reducing power (60.38 ± 0.78 µg/mL AAE) compared to the N-hexane extract (48.41 ± 1.78 µg/mL AAE). Overall, D. microcarpum shows potential as a natural antioxidant and supports its traditional medicinal use in treating gastrointestinal ailments, owing to its significant antimicrobial activity against clinical isolates.

Keywords: *Detarium microcarpum, phytochemicals, antioxidants, Bacteria*

Introduction

For millennia, plants have served as a natural source of medicine, offering a wealth of bioactive compounds with therapeutic properties (Lawal *et al.*, 2022). Investigating these plant-derived compounds has resulted in the discovery of new medications and treatments, establishing plants as an essential resource in the continuous effort to tackle global health challenges. Their natural origin and minimal side effects have made them popular in both developing and developed countries for millennia. It is estimated that about 75% of the world's population relies on plants or their extracts for healthcare (Abdulrahman, 2021). These plants are often used fresh, utilizing extracts from the entire plant or specific parts such as leaves, roots, flowers, or fruit. Additionally, woody components like bark and roots are also employed (Ogidi, 2023). With the upward surge of antimicrobial resistance among bacteria, medicinal plants offer a promising source of antimicrobial compounds (Jadimurthy *et al.*, 2023).

Detarium microcarpum, a member of the Fabaceae family, is commonly known as the tallow tree in English and referred to as "Taura" by the Hausa ethnic group in Northern Nigeria (Sanusi, 2020). This tree, typically found in savannah regions, can grow up to 9.114 meters tall and has reddish-brown, scaly bark. Various parts of *D. microcarpum* are noted for their medicinal properties (Agbo et al., 2020). Dogara (2016) reported that *D. microcarpum* exhibits antiviral, cytotoxic, antibacterial, and hypoglycemic activities.

Sanusi et al. (2022) found that the bark, leaves, and roots of *D. microcarpum* are widely used for their diuretic and astringent properties. Various scholars have noted the medicinal uses of this plant, including treatments for rheumatism, venereal diseases, urogenital infections, hemorrhoids, caries, biliousness, stomach aches, intestinal worms, diarrhea, dysentery, malaria, leprosy, and impotence through infusions or decoctions of the leaves, bark, and roots (Dassou *et al.*, 2023). The powdered bark decoction is used to treat sore throats, headaches, itching, back pain, painful menstruation, hypertension, itching, measles, and fatigue. Additionally, decoctions of the leaves or roots are utilized for treating paralysis, meningitis, fatigue, cramps, difficult deliveries, fainting, and convulsions (Sani *et al.*, 2014). The Hausa ethnic group in Northern Nigeria claims that an infusion of the stem bark of *D. microcarpum* alleviates gastrointestinal discomfort.

Antioxidants are crucial for protecting health, as they help mitigate the risk of chronic diseases like cancer and heart disease, supported by scientific findings (Martemucci *et al.*, 2023). These beneficial compounds are predominantly sourced from grains, fruits, and vegetables in nature. Plant-derived antioxidants such as vitamin C, vitamin E, carotenes, and phenolic acids are known to effectively lower disease risks. In diets, the majority of antioxidant compounds are derived from plant-based sources and are classified into different groups (Shen *et al.*, 2022).

Antioxidants are compounds that neutralize the harmful effects of free radicals in the body. Natural antioxidant substances found in cells include Superoxide dismutase, glutathione peroxidase, glutathione reductase, and thioredoxin thiols, which help counteract oxidative stress (Averill-Bates, 2023). Alpha-tocopherols, another type of antioxidant compound, work by inhibiting the spread of free radicals. Therefore, the primary aim of this study is to assess the phytochemical composition of *D. microcarpum* stem bark and evaluate its antibacterial potential and antioxidant activity.

MATERIALS AND METHODS

Sample collection

The stem-bark sample was collected from Yola South and then put into a polythene bag and labeled accordingly. It was taken immediately to the laboratory for preparation and analysis.

Preparation of Crude Extracts

The plant sample underwent a series of preparation steps: it was first washed with tap water and then rinsed thoroughly with distilled water before being dried at room temperature (25 °C) for several days. Once dried, it was finely powdered using a mortar and pestle, and the resulting powder was stored in a cleaned and sterilized container within a desiccator. About 400g of this powdered *D. microcarpum* stem bark was accurately weighed and subjected to maceration with 3 liters of 70% aqueous ethanol in a glass container. The maceration process lasted for 7 days with periodic shaking to enhance the extraction of bioactive compounds. Following maceration, the extract was filtered through Whatman filter paper (No. 1) to eliminate any solid particles and plant residues. The filtered liquid was then collected in a sterile container and concentrated using a rotary evaporator under reduced pressure and controlled temperature.

Fractionation Technique

Fractionation of the extract involved suspending 100g of the initial extract in 250 mL of water, followed by partitioning with N-hexane and chloroform using a separating funnel to obtain the N-hexane and chloroform fractions. Each solvent fraction was then evaporated using a rotary evaporator to remove the solvent, leaving behind the concentrated fractions. These fractions were subsequently stored at 4 °C until further analysis could be conducted.

Phytochemical Analysis

This was conducted according to the methods described by Abaka et al., 2024.

Test Organisms

S. aureus and *P. aeruginosa* strains were obtained from the stock culture maintained at the Department of Microbiology, Modibbo Adama University Yola, Adamawa State, Nigeria. These strains were confirmed through cultural and biochemical characterization and subsequently preserved in a nutrient broth (NB) medium in a

DOI: 10.5281/zenodo.12740667

refrigerator. The inoculation density for experiments was standardized to a cell concentration of 1.0 x 10^{-8} CFU/mL, equivalent to 5% of the McFarland standard (Abaka *et al.*, 2024).

Antibacterial sensitivity assay

The antibacterial activity of *D. microcarpum* extracts against bacterial isolates was evaluated using the agar well diffusion method, following the protocol described by Abaka et al. (2024). Sterile nutrient agar was poured into sterile Petri dishes and allowed to solidify. A sterile swab stick was dipped into a standardized bacterial inoculum and used to evenly spread the bacteria on the agar surface under aseptic conditions, ensuring proper labeling. The inoculated plates were then left undisturbed for 30 min to facilitate the adhesion of the organisms to the agar surface. Following this, four wells were aseptically bored into the agar using a sterile cork borer with a 6 mm diameter. These wells were filled with 0.2 mL of *D. microcarpum* extracts at concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL, respectively. Positive control wells received 0.2 mL of a 20 mg/mL Amoxicillin solution, while negative control wells received 0.1 mL of DMSO. The plates were allowed to dry and then incubated at 37 °C for 24 h. Following incubation, zones of inhibition around the wells were observed, measured, and recorded in millimeters.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The broth dilution method described by Dahiru et al. (2023) was used to evaluate the extracts. The extracts were diluted in nutrient broth at 10-fold concentrations. Each dilution received 0.1 mL of standardized bacterial inoculum. Negative control tubes without bacterial inoculation were also prepared. The tubes were incubated aerobically at 37 °C for 24 h. The Minimum Inhibitory Concentration (MIC) was determined as the lowest extract concentration that inhibited bacterial growth. For Minimum Bactericidal Concentration (MBC) determination, a loopful from each tube showing no growth in the MIC assay was transferred to fresh nutrient agar plates (Oxoid). These plates were then incubated at 37 °C for 24 h, and any subsequent growth was observed and recorded.

FTIR analysis

FTIR analysis followed the methodology outlined in Salim et al. (2021). Approximately 500 mg of finely powdered fraction was placed directly on the diamond crystal or light path for analysis of functional groups. Spectra were recorded using a Perkin-Elmer FTIR (model spectrum 100 series, USA) at ambient temperature, spanning wavenumbers from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. Each spectrum was an average of 4 scans with a scan speed of 0.2 $cm^{-1\ s-1.}$

Antioxidant Assay

Total antioxidant capacity

The antioxidant capacity of the plant extract was assessed following the method outlined by Proestos et al. (2013). A 0.1 ml portion of the sample solution, equivalent to 100 mg, was mixed with 1 ml of a reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. Methanol was used as a substitute for the sample in the blank. The tubes were sealed and heated in a boiling water bath at 95 °C for 90 min. After cooling to room temperature, the absorbance of each aqueous solution was measured at 695 nM.

Total reducing power

The reducing power was assessed following the protocol described by Haji et al. (2008). Different concentrations of the extract (0.75 ml) were mixed with phosphate buffer (0.2M, pH 6.6, 0.75 ml) and potassium hexacyanoferrate (K3Fe (CN)6) (1%, w/v, 0.75 ml). The mixture was then incubated at 50 °C for 20 minutes in a water bath. The reaction was halted by adding trichloroacetic acid (TCA) solution (10%, 0.75 ml) and subsequently centrifuged at 800 g for 10 minutes. The supernatant (1.5 ml) was combined with distilled water (1.5 ml) and ferric chloride solution (0.1%, w/v, 0.1 ml), and left to stand for 10 minutes. The absorbance of the reaction mixture was measured at 700 nm to determine its reducing power. Higher absorbance values indicate greater reducing power, expressed in terms equivalent to ascorbic acid.

Statistical Analysis

The results obtained were expressed as mean ± standard error of the mean (±SEM) and statistically evaluated using the Statistical Package for Social Sciences (SPSS) version 22 software by one-way analysis of variance followed by Tukey's multiple comparison tests at $p < 0.05$ level of significance.

RESULT

Table 1. Phytochemical Composition of N-hexane Fraction and Chloroform Fraction of *D. microcarpum Stembark*.

Phytochemical	HF	CF
Alkaloids	+	+
Saponins	+	+
Steroids	+	+
Glycosides	-	-
Terpenoids	-	-
Flavonoids	+	+
Phenols	+	+
Anthraquinones	-	-

+ = Present, - = Absent

DOI: 10.5281/zenodo.12740667

Table 2. Antibacterial activity of *D. microcarpum* against *S. aureus* and *P. aeruginosa*

Concentration (mg/mL)		Zone of inhibition (mm)			
		S. aureus		*P. aeruginosa*	
Control (Amoxicillin)	Extract	NHET	CHET	HXET	CHET
	100	17.90 ±0.27[a]	14.07 ±0.22	21.17 ±0.73	19.63 ±0.47
	50	14.13 ±0.29[a]	11.50 ±0.17	16.73 ±0.12	15.63 ±0.54
	25	10.33 ±0.24[a]	8.37 ±0.27	12.66 ±0.17[a]	10.20 ±0.17
	12.5	5.10 ±0.21	4.33 ±0.24	6.53 ±0.19[a]	5.30 ±0.30
20		40		40	

Values in the same row with a superscript are significantly ($p < 0.05$) higher than CHET for the same organism

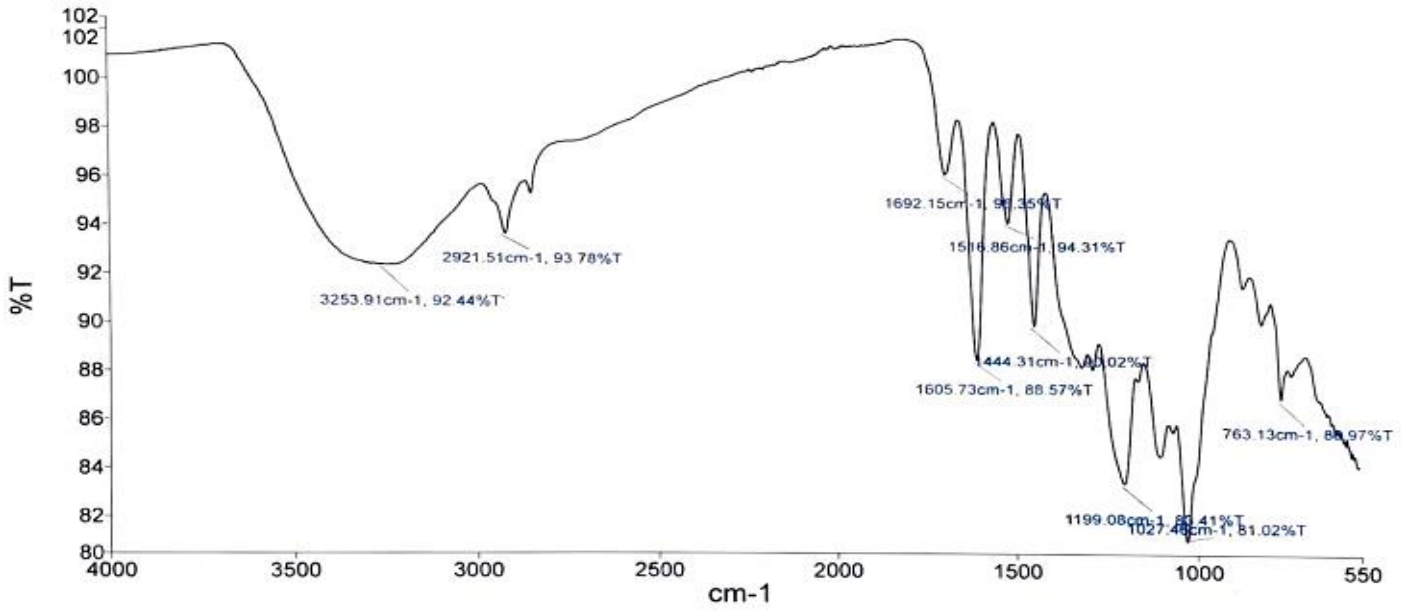
All values are significantly lower than the control at 20 mg/ml concentration.

Table 3. Minimum Inhibitory Concentration of *D. microcarpum* extracts against *S. aureus* and *P. aeruginosa.*

Organisms	Extract	Concentration (mg/mL)				
		100 mg/mL	50 mg/mL	25 mg/mL	6.25 mg/mL	3.125 mg/mL
S. aureus	NHET	-	-*	-	-	+
	CHET	-	-*	+	+	+
P. aeruginosa	NHET	-	-	-*	-	+
	CHET	-	-*	-	+	+

Table 4. Minimum Bactericidal Concentration of *D. microcarpum* extracts on test isolates

Organism	N-hexane extract (mg/mL)	Chloroform extract (mg/mL)
S. aureus	25	25
P. aeruginosa	25	25



DOI: 10.5281/zenodo.12740667

Figure 1. FTIR spectrum of the *n*-Hexane fraction of DM

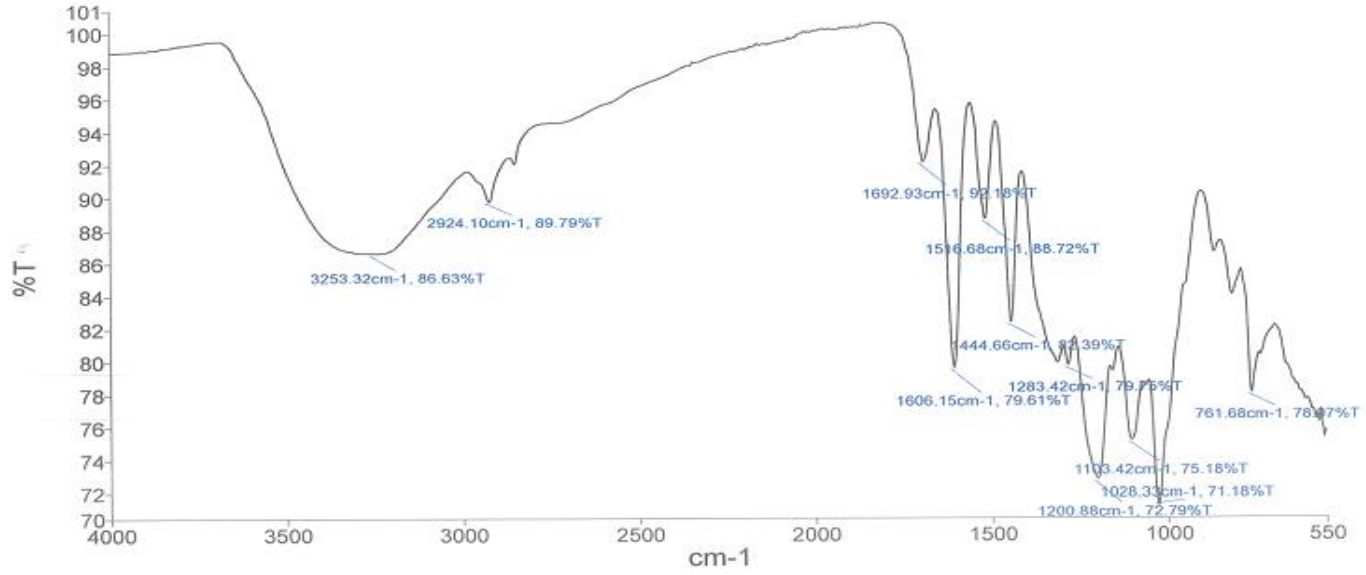


Figure 2. FTIR spectrum of the chloroform fraction of DM

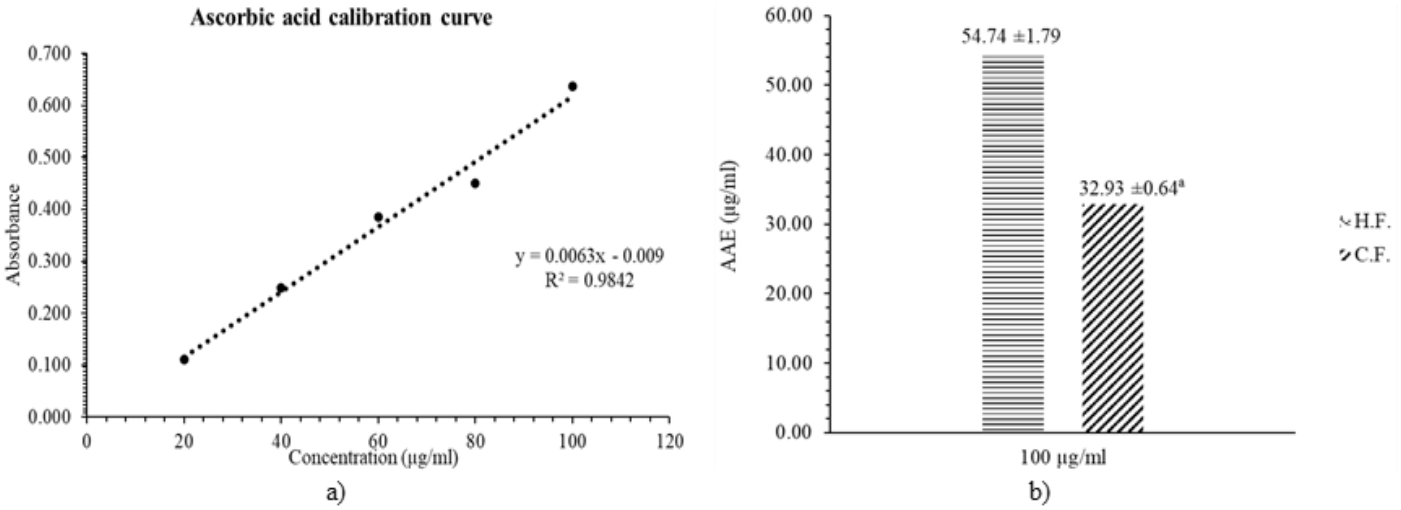


Figure 3. Total antioxidant capacity of DM: a) Ascorbic acid calibration curve and b) AAE total antioxidant capacity of DM. Value with [a] superscript is significantly ($p < 0.05$) lower than HF.

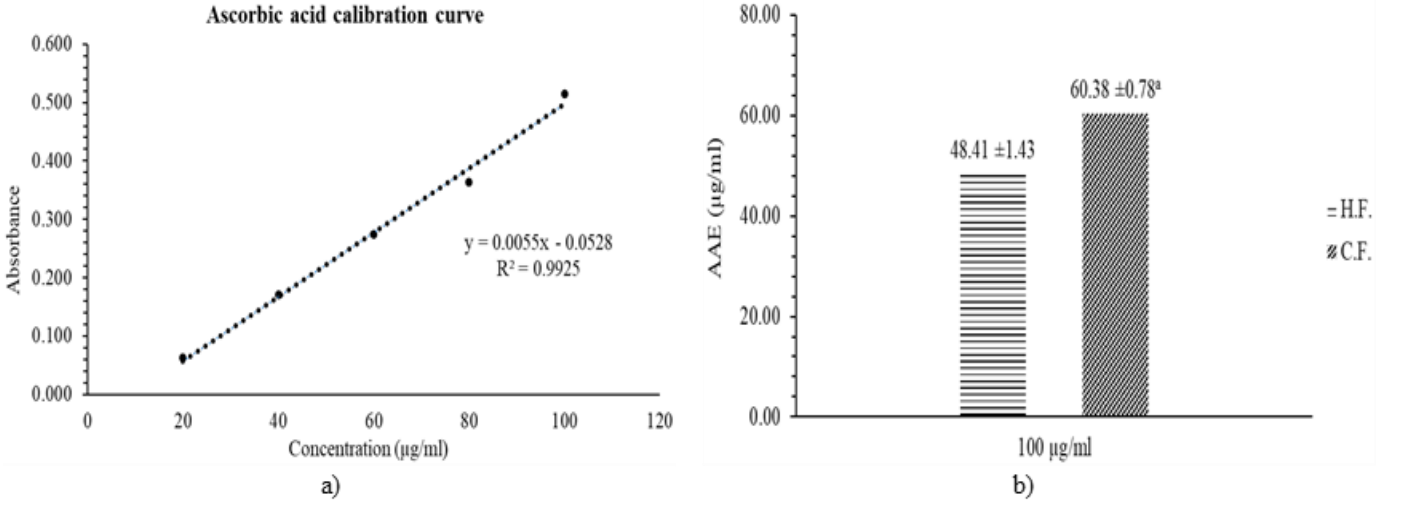


Figure 4. Total reducing power of DM: a) Ascorbic acid calibration curve and b) AAE total reducing power of DM. Value with [a] superscript is significantly ($p < 0.05$) higher than HF

DOI: 10.5281/zenodo.12740667

DISCUSSION

Phytochemical analysis of the n-hexane fraction (HF) and chloroform fraction (CF) of *Detarium microcarpum* revealed the presence of alkaloids, saponins, steroids, Phenols, and flavonoids in both fractions, while glycosides and terpenoids were absent in both (HF) and (CF) fractions (Table 4.1). These phytochemicals are known to play crucial therapeutic roles in managing various ailments, including oxidative stress (Engwa., 2018). This finding aligns with previous studies such as Okwu and Josiah (2006), which suggest that the therapeutic benefits of these metabolites present in *D. microcarpum* can be explored for medicinal purposes. The use of alcohol as an extraction solvent, as employed in this study, is recognized as a best practice for extracting broad-spectrum antimicrobial compounds (Ramasamy *et al.,* 2022). Steroids have been shown to influence oxidative stress by inhibiting DPPH-oxidase activity and activating the nuclear Nrf2 pathway, as reported by Arazi et al. (2017). In an experiment described by Mendonça et al., (2022), a well-known natural antioxidant was used as a positive control to evaluate the antioxidant activity of extracts. Evaluations of *D. microcarpum* using the DPPH radical scavenging assay method, as indicated by Meda et al. (2017), demonstrated a consistent increase in free radical scavenging activity across all extracts.

Plants inherently possess natural antioxidants that play a crucial role in cellular defense by scavenging reactive oxygen species (ROS), thereby offering protective effects against degenerative diseases in humans (Hassan et al., 2017). These antioxidants exert their effects through electron donation, neutralizing the harmful effects of free radicals generated during normal metabolic processes in aerobic cells (Sisein, 2014). This study explores the reducing power properties of various solvent extracts of *D. microcarpum*, considering multiple parameters. Plants rich in phenolic and polyphenolic acids, as highlighted by Shori (2015), often exhibit potent antioxidant and anti-radical activities. They can eliminate free radicals, activate antioxidant enzymes, chelate metal ions, reduce alpha-tocopherol radicals, and inhibit oxidases (Pisoschi *et al.*, 2021).

This group of compounds represents the primary class of natural antioxidants found in plants (Brewer, 2011). Plant flavonoids have been extensively studied for their antioxidant activity, both in vitro and in vivo (Shen *et al.,* 2022). They function by inhibiting the formation of reactive oxygen species, chelating trace elements involved in free radical production, scavenging reactive species, and protecting the cell's antioxidant defense mechanisms (Shen *et al.,* 2022). The ferric reducing ability (FRAP) of extracts, expressed in grams of ascorbic acid equivalent per gram of dried sample, reflects their reducing power, which correlates with antioxidant activity and serves as a significant indicator thereof (Proteggente *et al.,* 2002).

Figure 4.1 shows the FTIR spectrum of the *n*-Hexane fraction of *D. microcarpum* depicting the various peaks. The first absorption peak was at 3253.91 cm^{-1} corresponding to the O-H stretching frequency of the alcohol compounds. The second (2921.51 cm^{-1}) and third (1692.15 cm^{-1}) peaks at the frequency regions correspond to the N-H stretching frequency of amine salt and imine/oxime respectively while 1605.73 cm^{-1} corresponds to the C=C frequency of conjugated alkenes. In the fingerprint region, the peak at 1516.86 (N-O bending), 1444.31 (C-H bending), 1199.08 (C-O stretching), and 763.13 cm^{-1} (C-H bending) fingerprint regions correspond to the nitro compound, alkane, sulfonates, and monosubstituted alkane.

Figure 4.2 shows the FTIR spectrum of the chloroform fraction of DM depicting the various peaks. A total of 11 peaks were detected including four at the group frequency region. The first absorption peak was at 3253.32 cm^{-1} corresponding to the O-H stretching frequency of the alcohol compounds. The second (2924.10 cm^{-1}) and third (1692.93 cm^{-1}) peaks at the frequency regions correspond to the N-H stretching frequency of amine salt and imine/oxime respectively while 1606.15 cm^{-1} corresponds to the C=C frequency of conjugated alkenes. In the fingerprint region, 1516.68 (N-O bending), 1444.66 (C-H bending), 1283.42 (C-N stretching), 1200.88 (C-O bend), 1103.42 (C-O stretching), and 761.40 cm^{-1} (C-H bend) fingerprint regions corresponds to the amine, alkane, aromatic amine, alkyl aryl ether, secondary alcohol, and ortho-aromatics respectively.

The FTIR result depicts the presence of different functional groups in the *n*-hexane and chloroform fractions of *D. microcarpum* which might contribute to the pharmacological activities of the plants. Compounds possessing the amine groups were previously reported to exert anti-tumor activities correlating with previously reported anticancer effects (Li *et al.,* 2019). Furthermore, the antibacterial, antifungal, and antiviral activities of these compounds were reported (Hashem *et al.,* 2022). Sulfonates functional groups are important pharmaceutical intermediates used in drug synthesis with pharmacological properties including antiviral, antibacterial, and anti-inflammatory properties (Hu *et al.,* 2023). Compounds possessing alkane groups were previously reported to exert antimicrobial properties (Rhetso *et al.*, 2020).

Total antioxidant capacity is shown in Figure 4.3, the ascorbic acid (AA) calibration curve (a) and the AAE of the total antioxidant capacity of HF, and CF of *D. microcarpum* (b). The CF demonstrated a significantly ($p < 0.05$) lower total antioxidant capacity (32.93 ± 0.64 µg/mL AAE) than AF (54.74 ± 1.079 µg/mL AAE). The ascorbic acid calibration curve (a) and the AAE of the reducing power of HF and AF (b) are presented in Figure 4.4. The CF exhibited a significantly ($p < 0.05$) higher reducing power (60.38 ± 0.78 µg/mL AAE) than the HF (48.41 ± 1.78 µg/mL AAE).

D. microcarpum has been traditionally recognized for its healing properties, particularly when decoctions of its stem bark or roots are administered, as noted by traditional healers (Kolawole *et al.,* 2022). In this study, the stem bark extract demonstrated significant bactericidal effects at different concentrations. The variations observed in the effectiveness of these concentrations against different bacteria could potentially be attributed to the composition and types of fatty acids present in the extract, as discussed in studies by Yoon et al. (2018). Additionally, it was noted that more than 50% of the fatty acids identified in *D. microcarpum* consist of long and medium chains of unsaturated fatty acids, which have been reported to exhibit greater activity against gram-positive bacteria such as *S. aureus* and *P. aeruginosa* (Kolawole *et al.,* 2022).

Bacterial infections remain a significant global cause of mortality, prompting scientific evaluation of traditionally used plants for their antimicrobial properties and identification of active antibacterial compounds (Porras et al., 2020). Various parts of *D. microcarpum* have been traditionally employed for treating numerous infectious diseases, especially those of bacterial origin such as venereal diseases, urogenital infections, diarrhea, dysentery, leprosy, sore

DOI: 10.5281/zenodo.12740667

throat, and wound infections (Salawu et al., 2020). The plant extracts are generally utilized for both preventing and treating infections of diverse origins, supported by numerous studies documenting the antimicrobial activities of *D. microcarpum* extracts (Dogara, 2022).

The antibacterial evaluation of stem bark extracts from *D. microcarpum* across various concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL) against clinical isolates of *S. aureus* and *P. aeruginosa* demonstrated varying effectiveness. The N-hexane extract exhibited the highest zones of inhibition against *P. aeruginosa*, ranging from 21.17 ± 0.73 mm at 100 mg/mL to 6.53 ± 0.19 mm at 12.5 mg/mL. In contrast, for *S. aureus*, the inhibition zones were comparatively lower, measuring 17.90 ± 0.27 mm at 100 mg/mL and decreasing to 5.10 ± 0.21 mm at 12.5 mg/mL.

The chloroform extract of *D. microcarpum* also exhibited notable inhibition against *P. aeruginosa*, with the highest zone of inhibition at 19.63 ± 0.47 mm observed at 100 mg/mL and the lowest at 5.30 ± 0.30 mm at 12.5 mg/mL. Conversely, for *S. aureus*, the highest inhibition zone measured 14.07 ± 0.22 mm at 100 mg/mL, decreasing to 4.33 ± 0.24 mm at 12.5 mg/mL. The positive control, ciprofloxacin, consistently demonstrated inhibition zones of 40 mm for both bacterial strains. These findings indicate that both extracts are more effective against *P. aeruginosa* than *S. aureus*, with the N-hexane extract showing slightly higher efficacy. This outcome aligns with previous studies by Kolawole et al. (2022) and Sanusi et al. (2022), which highlighted the antibacterial activity of *D. microcarpum* against various bacteria including *E. coli*, *S. aureus*, *S. typhi*, *K. pneumonia*, and *P. aeruginosa*. Gram-positive bacteria generally exhibit higher susceptibility to extracts due to their simpler cell wall structure, characterized by a thick, porous peptidoglycan layer without an outer membrane. This structural difference allows antimicrobial agents easier access to their cellular targets. In contrast, Gram-negative bacteria possess a complex cell wall with an outer membrane containing lipopolysaccharides and proteins, which act as a significant barrier against antimicrobial substances, contributing to their higher resistance compared to Gram-positive bacteria.

According to Gera et al. (2016), the antibacterial activity observed in *D. microcarpum* indicates the presence of phytochemicals with growth-inhibiting properties, particularly flavonoids and tannins, known for their antibacterial effects. Despite traditional practices primarily using water for preparing plant extracts, studies have shown that organic solvents often yield extracts with higher antimicrobial activity compared to aqueous extracts (Sanusi *et al.*, 2022).

The superior activity of chloroform extracts compared to other extracts of different solvents, as noted by Tiwari et al. (2011), can be attributed to the polarity differences of the solvents used during extraction. These differences influence the extraction efficiency of bioactive compounds, resulting in varied levels of these compounds in the extracts.

The MIC values for the N-hexane and chloroform stem bark extracts of *D. microcarpum* are summarized in Table 4. The N-hexane extract exhibited an MIC of 50 mg/ml against *S. aureus* and 25 mg/ml against *P. aeruginosa* across all tested concentrations. Similarly, the chloroform extract showed an MIC of 50 mg/ml for both *S. aureus* and *P. aeruginosa*. These findings are consistent with previous studies by Dahiru et al. (2023) and Sunusi et al. (2022), which also reported an MIC value of 50 mg/ml for *S. aureus*.

Minimum Inhibitory Concentration (MIC) refers to the lowest concentration of an antimicrobial agent needed to prevent the growth of microorganisms. Clinically, MIC not only guides the dosing of antibiotics administered to patients but also aids in selecting appropriate antibiotics, thereby reducing the likelihood of microbial resistance to specific antimicrobial agents (Wiegand *et al.*, 2008). Variations in MIC values among different bacteria indicate that the effectiveness of an extract in inhibiting bacterial growth can be influenced by the unique characteristics and susceptibilities of each bacterial species (Abaka *et al.*, 2024).

The results in Table 5 indicate that the Minimum Bactericidal Concentration (MBC) for both the N-hexane and chloroform extracts against *S. aureus* and *P. aeruginosa* was determined to be 25 mg/mL. This finding aligns with a previous study by Sunusi et al. (2022), which reported similar results regarding the phytoconstituents and antibacterial effects of stem bark extracts of *D. microcarpum.* The variations in MBC values observed could be attributed to differences in the phytoconstituents of the extracts due to solvent variations, influencing their inhibitory and bactericidal effects compared to standard antibiotics (Abaka *et al.*, 2024).

Conclusion

Phytochemical analysis of *D. microcarpum* has identified bioactive compounds like flavonoids, phenolic acids, and tannins, indicating significant antioxidant properties that can mitigate oxidative stress. While promising in vitro results suggest health benefits, further in vivo studies and clinical trials are needed to confirm efficacy and bioavailability in humans. Understanding the mechanisms and potential synergistic effects among these compounds is essential. Overall, *D. microcarpum* shows potential as a valuable source of natural antioxidants, but more research is required to fully realize its therapeutic potential and ensure safety. In conclusion, *D. microcarpum* shows promise as a natural source of antioxidants based on its phytochemical profile. However, advancing these findings into practical applications requires ongoing research to establish its therapeutic potential and ensure its safe use.

The Chloroform extract showed the highest zones of inhibition against *P. aeruginosa* than *S. aureus* with the highest zone of inhibition at 19.63 ± 0.47 mm observed at 100 mg/mL and the lowest at 5.30 ± 0.30 mm at 12.5 mg/mL. Conversely, for *S. aureus*, the highest inhibition zone measured 14.07 ± 0.22 mm at 100 mg/mL, decreasing to 4.33 ± 0.24 mm at 12.5 mg/mL. The higher values of the chloroform extract suggest that the use of polar solvents as the extraction solvent is a better choice for the secondary metabolites present in the plant. The observed antimicrobial properties could be due to the presence of phytochemical constituents present in the extracts. The result of this study supports the traditional use of *D. microcarpum* in the treatment of diarrhea, dysentery, and other gastrointestinal ailments by the antimicrobial activity it has shown against the clinical isolates tested.

References

1. Abaka, A. M., Dahiru, M. M., Abubakar, K. B., Luka, J., Abubakar, A., Abdullahi, T. B., & Barau, S. H. (2024). Phytochemical Profile and Antibacterial Activity of Nigella Sativa against Biofilm-producing Bacteria

DOI: 10.5281/zenodo.12740667

Uropathogens. *Biology, Medicine, & Natural Product Chemistry*, *13*(1), 141-146.
2. Abdulrahman, M. D. (2021). Ethnomedicinal survey of plants used for management of inflammatory diseases in Ringim local government, Jigawa state, Nigeria. *Ethnobotany Research and Applications*, *22*.
3. Agbo, R. I., Vihotogbé, R., Missihoun, A. A., Dagba, R. A., Assogbadjo, A. E., & Agbangla, C. (2020). Indigenous knowledge of Detarium microcarpum Guill. & Perr.(Caesalpiniaceae) and implication for conservation in Benin (West Africa). *Environment, Development and Sustainability*, *22*, 6261-6285.
4. Arazi, H., Mohammadjafari, H., & Asadi, A. (2017). The use of anabolic androgenic steroids produces greater oxidative stress responses to resistance exercise in strength-trained men. *Toxicology reports*, *4*, 282-286.
5. Averill-Bates, D. A. (2023). The antioxidant glutathione. In *Vitamins and hormones* (Vol. 121, pp. 109-141). Academic Press.
6. Brewer, M. S. (2011). Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Comprehensive reviews in food science and food safety*, *10*(4), 221-247.
7. Dahiru, M. M., Abaka, A. M., & Artimas, S. P. (2023). Phytochemical Analysis and Antibacterial Activity of Methanol and Ethyl Acetate Extracts of Detarium Microcarpum Guill. & Perr. *Biology, Medicine, & Natural Product Chemistry*, *12*(1), 281-288.
8. Dassou, G. H., Favi, G. A., Salako, K. V., Jéronime, M. A. O., Trekpo, P., Akouete, P., ... & Adomou, A. C. (2023). An updated review of the African multipurpose tree species Detarium Senegalese JF Gmel.(Fabaceae). *South African Journal of Botany*, *157*, 525-539.
9. Dogara, A. M. (2022). Biological activity and chemical composition of Detarium microcarpum Guill. and Perr—a systematic review. *Advances in Pharmacological and Pharmaceutical Sciences*, *2022*(1), 7219401.
10. Engwa, G. A. (2018). Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases. *Phytochemicals: source of antioxidants and role in disease prevention. BoD–Books on Demand*, *7*, 49-74.
11. Haji, M. M., Hanifeh, M., Oveysi, M., Sadeghi, N., & Janat, B. (2008). Determination of total antioxidant capacity of green teas by the ferric reducing/antioxidant power assay.
12. Hashem, A. H., Hasanin, M., Kamel, S., & Dacrory, S. (2022). A new approach for antimicrobial and antiviral activities of biocompatible nanocomposite based on cellulose, amino acid, and graphene oxide. *Colloids and Surfaces B: Biointerfaces*, *209*, 112172.
13. Hassan, W., Noreen, H., Rehman, S., Gul, S., Amjad Kamal, M., Paul Kamdem, J., ... & BT da Rocha, J. (2017). Oxidative stress and antioxidant potential of one hundred medicinal plants. *Current topics in medicinal chemistry*, *17*(12), 1336-1370.
14. Hu, L., Jia, H., Zhang, J., da Silva-Júnior, E. F., Liu, C., Liu, X., & Zhan, P. (2023). Sulfonic acid: key drug design elements with potent, broad-ranging pharmacological activities. *Future Medicinal Chemistry*, *15*(22), 2029-2032.
15. Jadimurthy, R., Jagadish, S., Nayak, S. C., Kumar, S., Mohan, C. D., & Rangappa, K. S. (2023). Phytochemicals as invaluable sources of potent antimicrobial agents to combat antibiotic resistance. *Life*, *13*(4), 948.\
16. Kolawole, O. S., Musa, D., & Tukur, A. (2022). Antibacterial and phytochemicals status of Detarium microcarpum (guill and perr) stem bark. *Natural Resources for Human Health, (4): 385-390*
17. Lawal, I. O., Rafiu, B. O., Ale, J. E., Majebi, O. E., & Aremu, A. O. (2022). Ethnobotanical survey of local flora used for medicinal purposes among indigenous people in five areas in Lagos State, Nigeria. *Plants*, *11*(5), 633.
18. Li, Y., Xiao, J., Zhang, Q., Yu, W., Liu, M., Guo, Y., ... & Liu, Y. (2019). The association between anti-tumor potency and structure-activity of protein-kinase inhibitors based on quinazoline molecular skeleton. *Bioorganic & Medicinal Chemistry*, *27*(3), 568-577.
19. Martemucci, G., Portincasa, P., Centonze, V., Mariano, M., Khalil, M., & D'Alessandro, A. G. (2023). Prevention of oxidative stress and diseases by antioxidant supplementation. *Medicinal Chemistry*, *19*(6), 509-537.
20. Meda, N. R., Fraisse, D., Gnoula, C., Vivier, M., Felgines, C., & Senejoux, F. (2017). Characterization of antioxidants from Detarium microcarpum Guill. et Perr. leaves using HPLC-DAD coupled with pre-column DPPH assay. *European Food Research and Technology*, *243*, 1659-1666.
21. Mendonça, J. D. S., Guimarães, R. D. C. A., Zorgetto-Pinheiro, V. A., Fernandes, C. D. P., Marcelino, G., Bogo, D., ... & Nascimento, V. A. D. (2022). Natural antioxidant evaluation: A review of detection methods. *Molecules*, *27*(11), 3563.
22. Ogidi, O. I. (2023). Sustainable utilization of important medicinal plants in Africa. In *Sustainable Utilization and Conservation of Africa's Biological Resources and Environment* (pp. 323-351). Singapore: Springer Nature Singapore.
23. Okwu, D. E., & Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology*, *5*(4), 357-361.
24. Pisoschi, A. M., Pop, A., Iordache, F., Stanca, L., Predoi, G., & Serban, A. I. (2021). Oxidative stress mitigation by antioxidants-an overview of their chemistry and influences on health status. *European Journal of Medicinal Chemistry*, *209*, 112891.
25. Porras, G., Chassagne, F., Lyles, J. T., Marquez, L., Dettweiler, M., Salam, A. M., ... & Quave, C. L. (2020). Ethnobotany and the role of plant natural products in antibiotic drug discovery. *Chemical Reviews*, *121*(6), 3495-3560.
26. Proestos, C., Lytoudi, K., Mavromelanidou, O. K., Zoumpoulakis, P., & Sinanoglou, V. J. (2013). Antioxidant capacity of selected plant extracts and their essential oils. *Antioxidants*, *2*(1), 11-22.
27. Proteggente, A. R., Pannala, A. S., Paganga, G., Buren, L. V., Wagner, E., Wiseman, S., ... & Rice-Evans, C. A. (2002). The antioxidant activity of regularly consumed

DOI: 10.5281/zenodo.12740667

fruits and vegetables reflects their phenolic and vitamin C composition. *Free radical research*, *36*(2), 217-233.

28. Ramasamy, S. P., Rajendran, A., Pallikondaperumal, M., Sundararajan, P., Husain, F. M., Khan, A., ... & Alqasim, A. (2022). Broad-spectrum antimicrobial, antioxidant, and anticancer studies of leaf extract of Simarouba glauca DC in vitro. *Antibiotics*, *11*(1), 59.
29. Rhetso, T., Shubharani, R., Roopa, M. S., & Sivaram, V. (2020). Chemical constituents, antioxidant, and antimicrobial activity of Allium chinense G. Don. *Future Journal of Pharmaceutical Sciences*, *6*, 1-9.
30. Salawu, K. M., Wang, Y., Maharjan, R., & Ajaiyeoba, E. O. (2020). Antibacterial screening and isolation of compounds from Detarium microcarpum stem bark against methicillin-resistant Staphylococcus aureus. *Journal of Science and Practice of Pharmacy*, *7*(1), 400-405.
31. Sani, A., Agunu, A., Danmalam, H. U., & Ibrahim, H. (2014). Pharmacognostic studies of the stem bark of Detarium microcarpum. *Guill and Perr.(Fabaceae). Natural Products Chemistry and Research*, *4*, 2-8.
32. Sanusi, S. B., Lawal, S. M., Usman, A., Musa, F. M., Ardo, B., & Way, T. B. (2022). Phytochemical analysis and antibacterial activity of stem bark extracts of deuterium microcarpum against bacteria causing gastrointestinal tract infections in humans. *Dutse Journal of Pure and Applied Sciences*, *8*(1b), 82-89.
33. Shen, N., Wang, T., Gan, Q., Liu, S., Wang, L., & Jin, B. (2022). Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. *Food Chemistry*, *383*, 132531.
34. Shori, A. B. (2015). Screening of antidiabetic and antioxidant activities of medicinal plants. *Journal of Integrative Medicine*, *13*(5), 297-305.
35. Sisein, E. A. (2014). Biochemistry of free radicals and antioxidants. *Scholars Academic Journal of Biosciences*, *2*(2), 110-118.
36. Tiwari, S. K. (2011). *Extraction, Phytochemical Study and Evaluation for Biological Activities of Cassia Auriculata Leaves* (Master's thesis, Rajiv Gandhi University of Health Sciences (India)).
37. Yoon, B. K., Jackman, J. A., Valle-González, E. R., & Cho, N. J. (2018). Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. *International journal of molecular sciences*, *19*(4), 1114.

DOI: 10.5281/zenodo.12740667