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RESEARCH ARTICLE

Antimicrobial Potential of *Combretum molle* Leaf Extracts: Insights from Zambia

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Abstract

The global rise in antimicrobial resistance (AMR) necessitates innovative solutions. This study investigates the antimicrobial activity and phytochemical composition of *Combretum molle*, a native Zambian plant, to explore its potential as a cost-effective source for novel antimicrobial agents. Exhaustive extraction using solvents of varying polarities was performed on dried leaf extracts of *C. molle*. These extracts were tested against a panel of clinically significant gram-negative and gram-positive bacterial isolates and screened for phytochemicals. Phytochemical screening revealed the presence of steroids, terpenoids, phenols, flavonoids, tannins, saponins, alkaloids, and glycosides. Antimicrobial activity was assessed using disk diffusion and broth microdilution techniques, with minimum inhibitory concentrations (MICs) determined through spectrophotometry and spot inoculation. The results revealed substantial antimicrobial activity, with hexane extracts showing the highest efficacy (MIC range: 3.6 to 50.4 mg/ml) and ethanol extracts exhibiting comparable activity to tetracycline (inhibition zones: 6 to 26 mm, P-value < 0.05). These findings highlight the therapeutic potential of *C. molle* extracts and support their possible integration into ethnomedicine as alternatives to conventional antimicrobials. By tapping into nature's arsenal, this study contributes to the search for effective strategies against multi-drug resistance, offering hope in the fight against AMR.

Keywords: Antimicrobial Resistance; *Combretum molle*; Natural Antimicrobials; Phytochemical Screening, Bacteria, Ethnomedicine

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1.0 Introduction

The escalating threat of antimicrobial resistance (AMR) poses a formidable challenge to global public health, complicating the efficacy of conventional therapies [1]. Antimicrobial Resistance evolves through selective pressure from widespread antimicrobial usage in agricultural, veterinary, and clinical domains [2,3]. Despite available antimicrobial agents, the emergence of resistant and multi-drug-resistant strains persists, and it remains a serious challenge for public health and veterinary medicine[4]. Conversely, antimicrobial drug discovery and production have failed to match the pace of antibiotic resistance, emphasizing the urgent need for alternative therapeutic strategies [5]. Key pathogens contributing to the AMR crisis, are those belonging to the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter species*) group of bacteria which are usually multi-drug resistant in nature [6,7]. Most of these bacteria are multidrug resistant, which is one of the greatest challenges in clinical practice. Multidrug resistance is amongst the top three threats to global public health [7]. There are a number of examples of pathogens that tend to drive multidrug resistance such as *S. agalactiae* a zoonosis that has been implicated in neonatal infections and has been known for increasing resistance to erythromycin and clindamycin [8,9]. *P. aeruginosa*, notorious for hospital-acquired infections has been known to exhibit intrinsic and acquired resistance mechanisms [10] and it is also multi drug resistant [11]. *E. coli*, a common urinary tract pathogen, has shown resistance and been linked to the emergence of extended-spectrum beta-lactamase (ESBL) and carbapenemase-producing strains, complicating treatment [12]. Methicillin-resistant *S. aureus* (MRSA) continues to pose a global healthcare challenge [13], while *K. pneumoniae* increasingly resists carbapenems thereby complicating therapy [14]. *Acinetobacter* species, notably *Acinetobacter baumanii*, rapidly develops multidrug resistance and it is one of the WHO priority pathogens [15]. Although there are fewer studies on *Aeromonas* bacteria, it has been widely agreed that its strains contribute to various infections and exhibit antimicrobial resistance, necessitating surveillance [16].

Traditional medicine remains a primary healthcare resource for 70-80% of the world's population especially rural people in developing countries [17]. *Combretum molle* (*C. molle*), from the Combretaceae family, English name “Velvet Bushwillow”, locally known as 'Mulama' in Northwestern Zambia, is a deciduous tree or shrub, known for its velvety foliage and yellowish-green flowers. It holds traditional medicinal significance, in Southern Africa, among indigenous healers, who have attested to its efficacy in managing arthritis and other inflammatory conditions as well as respiratory infections [18]. Previous studies have explored various medicinal uses of *C. molle*, with extracts demonstrating antimicrobial, antioxidant, and anti-inflammatory properties [18–20]. Despite its pharmacological potential, no comprehensive studies have been conducted in Zambia to elucidate the therapeutic properties of *Combretum molle*, particularly in addressing antimicrobial resistance (AMR). Little is known about the role such beneficial potential can play in addressing the shortcomings of current therapies and drug candidates indicated against AMR strains. In this study bacteria were purposively selected as a result of their clinical impact and varying resistance profiles observed from previous studies [21]. The bacteria included, *Streptococcus agalactiae (S. agalactiae)*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter*, and *Aeromonas*.

2.0 Materials and Methods

2.1 Selection and Collection of Plant Material

Leaf samples of *Combretum molle* were harvested following a non-participatory rapid appraisal from the vicinity of Kalumbila District at Kayonge (-12.3944925, 26.0434206), North Western Province, Zambia. Plant identification was conducted by botanists at the University of Zambia, Biological Sciences Departmental herbarium, and voucher specimens were deposited. The leaves were washed, dried at 35℃ in a Yamato® DG-81 Drying oven for 10 days, and ground to coarse powder using a mechanical grinding mill (Salton Grinding Mill, SB420E).

2.2 Extraction Process

Dried and ground *C. molle* (5g) was subjected to exhaustive extraction with hexane, hexane-ethyl acetate (50:50), dichloromethane, and ethanol, sequentially as methodically established by Harborne [21]. Briefly, 5 grams of the plant material was mixed with 100 ml of solvent in a conical flask. The mixture was sonicated for 15 minutes at room temperature in an ultrasound water bath (Elmasonic S 40 H, 37 kHz, 140 W; Elma, Germany), and left to stand in the dark at room temperature for 24-hours with interval shaking. Thereafter, the mixture was filtered with Whatman No. 4 filter paper. The process was repeated twice for each solvent and before moving to the next solvent, the residue was dried in an oven at 40 ℃. Extracts were concentrated under reduced pressure using a rotavapor (BUCHI R-II, Switzerland) at 40℃, and stored at 4 ℃ in labelled amber containers for further bioassay.

For preliminary disk diffusion assays, only ethanol was used for maceration, shaken for 6 hours, and allowed to stand for 18 hours, followed by vacuum freeze-drying (Yamato Neocool, 221420, Tokyo, Japan) at 24℃. The resulting extract was re-dissolved in Dimethylsulfoxide (DMSO).

2.3 Preparation of Bacterial Isolates

Two panels of bacterial strains were employed in this study. The first panel utilized preliminary disk diffusion assays, which comprised *Staphylococcus aureus*, *Escherichia Coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter spp.* and *Aeromonas spp* field isolates. The second panel employed broth microdilution assays, that included field strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus agalactiae* and *Pasteurella multocida*, along with the *Staphylococcus aureus* ATCC control strain ST 00886. Field strains were preferred due to their higher levels of resistance relative to control strains [22,23]. All bacterial species were isolated from clinical samples submitted to the University of Zambia, School of Veterinary Medicine Microbiology laboratory for bacterial disease diagnosis and have been documented in previous reports [24,25].

2.4 Antimicrobial Assays

2.4.1 Kirby-Bauer Disk diffusion

The antibiotic susceptibility testing was piloted as a subset of a larger study using the disc diffusion method of Kirby and Bauer [26,27] on Mueller-Hinton (MH) agar (Oxoid, Germany) based on the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2009). Inocula were prepared from resuscitated subcultures of bacteria and standardized to 1.5×10^8 CFU/ml. Mueller Hinton agar plates were inoculated with standardized bacterial suspensions, and qualitatively prepared antibiotic disks with 30µg absorbed plant extracts (421.5mg/ml (w/v)) were placed on the plates. The plates were incubated at 37℃ for 24 hours, and zones of inhibition were measured. Tetracycline (30µg) disks served as positive controls, while 100% DMSO was used as a negative control.

2.4.2 Broth Microdilution

The experiment was carried out in quadruplicates to cater for all the solvents, utilizing well-established techniques [28]. In summary, Mueller Hinton Broth (MHB) (Oxoid, UK), was dispensed (200µL) to every well of a 96-well microtiter plate. Subsequently, plant extracts (50µL) were serially diluted into each well, excluding the control wells, with the hexane fraction containing 9 mg, hexane+ethyl acetate 3.8 mg, dichloromethane 3.5 mg, and ethanol 19.9 mg per 50µL. Bacterial suspensions (1.5×10^8 CFU/ml, 0.5 McFarland standard) were added to the wells, and the plates were incubated at 37 ℃ for 20 hours. Absorbance was measured pre- and post-incubation at 600 nm (Thermo Scientific Multiskan FC), and minimum inhibitory concentrations were determined.

2.5 Determination of Bactericidal or Bacteriostatic Effects

Wells indicating susceptibility were selected for spot inoculation onto Nutrient Agar (Oxoid, UK) plates and incubated at 37 ℃ for 24 hours in a time-kill test [29]. Plates were observed for bacterial growth after an additional 48-hour incubation at room temperature.

2.6 Phytochemical analysis

Phytochemical screening of the *Combretum molle* leaf extracts was conducted qualitatively following standard protocols [30,31] . In brief, preparation of the stock solution involved dissolution of 0.5 g of sample extracts from each solvent in 40 ml of 70% methanol. For the tannins/phenols test, 3 drops of 5% ferric chloride were added to 2 ml of plant extract, resulting in a transient greenish to dark bluish color. Saponins were identified by vigorously shaking 5 ml of each plant extract with 5 ml of distilled water, forming a stable foam. Flavonoids were detected by adding 2 ml of 2% sodium hydroxide to 2 ml of plant extract, which turned yellow and colorless upon addition of dilute hydrochloric acid. The presence of alkaloids was confirmed with Mayer's test, producing a creamy or white precipitate upon adding Mayer's reagent to the extract. Glycosides were detected by mixing 5 ml of the plant extract with 2 ml of glacial acetic acid, 2 drops of 5% ferric chloride, and 2 ml of concentrated sulfuric acid, resulting in a greenish-bluish color in the lower layer. Terpenoids were identified using the Salkowski test, where 3 ml of plant extract solution was mixed with 2 ml of chloroform and 2 ml of concentrated sulfuric acid, forming a reddish-brown color at the interface. Finally, steroids were detected by adding 3 ml of chloroform to 2 ml of plant extract, followed by concentrated sulfuric acid, resulting in a reddish-brown ring.

2.7 Statistical analysis

Data collation and analysis was performed using MS Excel Professional Plus 2019, version 1808. The paired t-test was employed to determine statistically significant differences in zone diameters between plant extracts and antibiotics, with a significance level set at 0.05 [32].

2.8 Ethical approval

Approval number 3666-2023 was granted for this study by the University of Zambia Biomedical Research Ethics Committee (IRB00001131 of IORG0000774) and registered by the Zambia National Health Research Authority (NHRAR-R-234/19/03/2023). All infectious materials were handled and disposed of in accordance with the set Good Laboratory Practices of the University of Zambia, School of Veterinary Medicine, Microbiology Unit.

3.0 RESULTS

In this study, a solvent based panel of extracts of *C. molle* was assessed for antimicrobial activity against select antimicrobial-resistant (AMR) priority pathogens. We commenced by quantifying the extract yield from each of the four solvents used for the exhaustive extraction that would facilitate subsequent dilutions. Thereafter, we investigated the antimicrobial activity of the various solvent extracts of the *Combretum molle* leaf powder and qualitatively determined the phytochemical composition of the extracts. The extracts' bioactivity was assessed against WHO priority list AMR bacterial pathogens to determine comparative susceptibility [33].

3.1. Extract yield

Table 1 presents the exhaustive extraction yields of *C. molle* leaf powder using four solvents. Ethanol demonstrated the highest yield, producing 2390 mg of extract, whereas dichloromethane yielded the lowest at 140 mg from 5 g (5000 mg) of plant material.

Table 1. Extract yield of *C. molle* leaf powder (5g) Exhaustively extracted with four solvents.

Solvent	Mass extracted (mg)	Mass extracted (g)	Stock Solution Conc^{n} (mg/mL)	Total Mass Yield %

Hexane	540	0.54	180	10.8
Hexane+Ethyl Acetate	380	0.38	76	7.6
Dichloromethane	140	0.14	70	2.8
Ethanol	2390	2.39	398	47.8

Conc^{n}, Concentratrion.

3.2 Antimicrobial Activity

The antimicrobial activity of the extracts was evaluated using Kirby-Bauer Disk Diffusion and Broth Microdilution Method.

3.2.1 Kirby-Bauer Disk Diffusion

The data from the piloting trial using the disk diffusion method indicated in Table 2, presents the spectrum of observed zones of inhibition, ranging from 6 to 26 mm. A stringent criterion of ≥ 11 mm was established to denote bacterial susceptibility, applied uniformly to both the extracts and the positive control antibiotic, tetracycline (30 µg), which displayed inhibition zone diameters spanning 14 to 23 mm. Notably, *Staphylococcus aureus* exhibited remarkable susceptibility to the extract, while *Klebsiella pneumoniae* demonstrated pronounced resistance. However, statistical analysis, using a Paired T-test, revealed no statistically significant differences (t Stat = -0.24643, P(T<=t) two-tail = 0.815145, t Critical two-tail = 2.570582) in inhibition zone diameters between the plant extracts and the antibiotic.

Table 2. Zones of inhibition (mm) of the extract, tetracycline and dimethyl sulfoxide against *Staphylococcus*, *E. coli*, *Pseudomonas*, *Klebsiella*. *Acinetobacter* and *Aeromonas*.

Treatment	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. Pneumoniae*	*Acinetobacter*	*Aeromonas*
Combretum molle	26	22	18	6	14	16
Tetracycline (30µg)	23	18	18	16	14	16
DMSO (100%) (Negative Control)	6	6	6	6	6	6

3.2.2 Broth Microdilution Method

Figure 1 (a and b) illustratively provides, using the ethanolic extracts for exemplification, the antimicrobial activity of *C. molle* relative to the positive control (cefotaxim) against; *S. aureus*, *P. aeruginosa*, *E. coli*, *Streptococcus*, and *P. multocida*, resulting from the broth microdilution assays in 96 well plates. Notably, *P. multocida* displayed high susceptibility to both the extracts and the positive control. The chart indicates cross cutting resistance to the exhaustive ethanol extract and some varying levels of resistance to the antibiotic. The susceptibility was graded using the CLSI grading system [34]

3.2.2.1. Spectrophotometric Assessment of Antimicrobial Activity: Illustrative Observations Before and After Incubation

Figure 3 (a and b) showcases typical spectrophotometric findings indicating fluctuations in absorbance, correlating with shifts in viable cell count, pre- and post-incubation. Generally, the absorbance for all bacterial strains increased with lower extract concentrations. The similar pre and post incubation absorbances at higher concentrations are attributable to the dark colour of the leaf extract. Due to the

limitation of using absorbance alone to definitively determine bacterial growth or death, colony-forming unit (CFU) counting through swabbing of wells and spot inoculation onto Nutrient Agar (Oxoid, UK), was employed for confirmation and to determine the Minimum Inhibitory Concentration.

3.2.2.2. Minimum Inhibitory Concentration (MIC) of Extracts

Table 3 presents the Minimum Inhibitory Concentration (MIC) results, revealing the superior antimicrobial efficacy of the hexane extract, achieving an MIC as low as 3.6 mg/ml. Following this, the hexane + ethyl acetate combination exhibited an MIC of 5.32 mg/ml, while dichloromethane and ethanol displayed MICs of 9.8 mg/ml and 55.7 mg/ml, respectively. The MIC values for cefotaxime, serving as the positive control, were not directly determined but referenced from EUCAST [35] , where they ranged from 0.0005 to 0.064 mg/ml.

Table 3. Zones of inhibition (mm) of the extract, tetracycline and dimethyl sulfoxide against *Staphylococcus*, *E. coli*, *Pseudomonas*, *Klebsiella*. *Acinetobacter* and *Aeromonas*.

Isolate ID	Hexane (mg/ml)	Hex+EtOAc (mg/ml)	DCM (mg/ml)	Ethanol (mg/ml)
*S. aureus*_P3-8	12.6	21.28	9.8	111.44
*S. aureus*_ST 00886	3.6	10.46	9.8	111.44
Streptococcus	12.6	21.28	9.8	55.72
E. coli	25.2	21.28	9.8	111.44
P. aeruginosa	25.2	10.46	19.6	111.44
*S. aureus*_PM7	25.2	10.46	9.8	111.44
*S. aureus*_PM81-1	50.4	5.32	9.8	111.44
P. multocida	12.6	10.46	9.8	111.44

Hex, Hexane; EtOAc, Ethyl Acetate; DCM, Dichloromethane; CTX, Cefotaxim

3.3 *Bactericidal or Bacteriostatic Effects of C. molle Extract*

In Table 4, the time-kill susceptibility observations of the isolates were meticulously recorded, serving to discern the bactericidal or bacteriostatic properties of the extracts [29]. This assessment method not only evaluated the efficacy of the extracts but also elucidated their impact on bacterial growth dynamics. The tests conducted indicated no change in activity from susceptible, suggesting a potential bactericidal effect of the *C. molle* extract.

Table 4. Time-kill assessment for bactericidal or bacteriostatic activity

ID of Isolate & Well Swabbed	Hexane Fraction	Hexane +Ethyl Acetate Fraction
*S. aureus*_P3-8 (A1)	S	ND
*S. aureus*_ ST 00886 (B1)	ND	S
*S. aureus*_ ST 00886 (B3)	S	ND
Streptococcus (C1)	S	ND
E. coli (D1)	S	ND
P. aeruginosa (E1)	S	S
*S. aureus*_PM7 (F1)	S	ND

*S. aureus*_PM81-1 (G2)	S	S
P. multocida (H1)	S	ND

S= Susceptible, ND= Not Determined

3.4. *Qualitative Phytochemical Screening*

The qualitative phytochemical analysis of the sequential fractional exhaustive extracts of *Combretum molle* leaves, as shown in Table 5, revealed that hexane primarily extracted steroids and terpenoids (++), with moderate presence of saponins (+). More polar solvents, such as ethanol, extracted a broader range of bioactive compounds, including phenols, flavonoids, tannins, and saponins (++), with minimal extraction of alkaloids and glycosides in hexane but notable presence in dichloromethane and ethanol extracts. The ethanol extract which had the highest extractive value (w/w), could be indicative of the richer content of polar compounds.

Table 5. Qualitative phytochemical analysis of *C. molle* fractions

Phytochemical	Hexane Extract	Hex & Ethyl Acetate	Dichloromethane	Ethanol Extract
Alkaloids	-	-	+	+
Phenols	-	+	+	++
Flavonoids	-	+	+	++
Tannins	-	+	+	++
Steroids	++	+	-	+
Saponins	+	+	+	++
Terpenoids	++	+	-	+
Glycosides	-	-	+	+

+ Present; ++ Present in high amounts; - Absent

4.0 DISCUSSION

The escalating resistance to conventional antibiotics underscores the urgency to explore alternative, efficient, and cost-effective approaches for combating infectious diseases [36,37]. Previous studies have documented the antimicrobial activity of *C. molle* against bacteria, fungi, and helminths [18,38,39]. However, the bioactivity of *C. molle* within the Zambian ecological zone against selected test organisms remains unexplored. Hence, this study aimed to evaluate the antimicrobial potential of *C. molle* leaf extracts against critical case-selected test organisms.

Our investigation highlighted the effectiveness of ethanol and hexane as solvents for extraction, resulting in substantial yields of 2390 mg (47.8%) and 540 mg (10.8%) respectively, whereas dichloromethane yielded the least at 140 mg (2.8%). Our extraction method, which involved sequential extraction, allowed each solvent to target compounds not extracted by the previous solvent. This process was predicated on the similarity in polarities between the compounds and solvents. Therefore, while ethanol yielded a higher crude extract quantity, the ultimate determinant of efficacy lies in the bioactivity of the extracts, which may not necessarily correlate with yield.

Although previous studies [38–41], have reported varied yields, the observed higher yield with ethanol extraction aligns with the ability of ethanol to extract both polar and nonpolar molecules [42].

Our pilot study, which was a subset of a larger pilot investigation involving 144 discs for the antimicrobial assay demonstrated comparable antibacterial activity of the ethanol extract with the standard antibiotic tetracycline ($p < 0.05$). This suggests a potential broad-spectrum effect across Gram-positive and Gram-negative bacteria. It's important to note the limitations of a pilot trial within the

broader context of the study. Notably, our findings contradict the common notion that plant extracts are more effective against Gram-positive bacteria due to the impermeable nature of Gram-negative bacteria's outer membrane [43–45]. However, due to the exploratory and piloting nature of this part of our study, further larger-scale investigation to establish the breadth of antibacterial activity of the ethanol extract may be warranted.

Following exhaustive extraction, the hexane extract emerged as the most active fraction relative to the other solvent fractions, which is consistent with previous findings demonstrating varied efficacy across different extracts [39,41]. Notably, the susceptibility of the ATCC control strain for *S. aureus* (ST 00886) justifies the preference for field strains over control strains in drug discovery and resistance studies [46,47].

The observed patterns in absorbance readings pre- and post-incubation corresponded with changes in viable cell count, validating our optical density readings as a measure of microbial growth or inhibition [48]. Importantly, our study suggests that the effects of *C. molle* extracts may be broad-spectrum and bactericidal, warranting further dose-dependent assessments.

Collaborative ethnoveterinary and ethnomedical research involving indigenous knowledge holders, chemists, veterinary and medical scientist's indispensable in providing valuable insights into medicinal herbs during this study. This interdisciplinary approach facilitates evidence-based interventions, proven effective in numerous studies [36,49–51]. While most studies have revealed the antimicrobial effects of *C. molle* extracts, no known studies have expounded on whether the activity is bactericidal or bacteriostatic [52]. The consistency in susceptibility patterns observed in this study suggest for the first time that the effects of the *C. molle extracts* may be broad spectrum and bactericidal as both gram negative and gram-positive isolates were inhibited. However, further dose-dependent assessments may be needed to affirm this suggestion. While a report [39] highlighted the high potency of acetone extracts from *C. molle* bark, our exhaustive extraction using a spectrum of low to high polarity solvents similarly revealed the activity of the leaf ethanol extracts. This finding could be attributable to ethanol's slightly higher polarity index (5.2) compared to acetone (5.1) [53,54]. Despite the historical emphasis on acetone extracts, our results suggest that ethanol extracts may be equally effective. This contrast underscores the versatility of solvent extraction methods and highlights the importance of exploring different solvents to maximize the extraction of bioactive compounds from plant materials. Further investigation into the specific chemical composition and antimicrobial properties of both ethanol and acetone extracts is warranted to elucidate the underlying mechanisms of their efficacy.

The strong antimicrobial activity of the hexane extract, despite its higher yield of non-polar phytochemicals like steroids and terpenoids, highlights the significant bioactivity of these constituents. Conversely, the ethanol extract's higher extractive value and diverse range of bioactive compounds, including phenols, flavonoids, and tannins, underscore its potential for broader pharmacological applications. The correlation between solvent polarity and the types of phytochemicals extracted provides insight into optimizing extraction methods for desired bioactive compounds, demonstrating the complementary roles of different extracts in harnessing the full therapeutic potential of *Combretum molle* [39,55].

4.0 Discussion

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Conclusion

This study underscores the *in vitro* antimicrobial activity of crude *C. molle* extracts, supporting their adjunct use in combating antimicrobial resistance from a Zambian perspective. The plant holds promise for providing novel or lead compounds, potentially serving

as starting materials for new drug synthesis. Further investigations to isolate and characterize active compounds are imperative. Additionally, establishing its safety profile and implementing conservation efforts are needed to protect this species and its habitats from overexploitation and degradation.

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APPENDICES: FIGURES

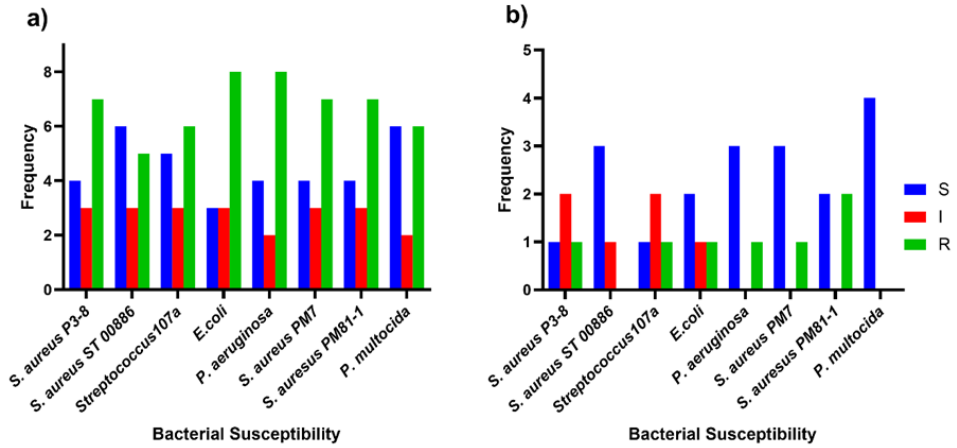

Figure 1. (a) Antimicrobial Activity of *C. molle* ethanolic extracts at 28% to 3.5% serial dilutions &; (b) Cefotaxim (control) Antimicrobial Activity on the test organisms (S, Susceptible; I, Intermediate Susceptible; R, Resistant). Figure 2 below provides a visual summary of the susceptibility profile of the extracts according to the extracting solvent. The hexane fraction showed notable antimicrobial activity, while the hexane + ethyl acetate fraction exhibited resistance.

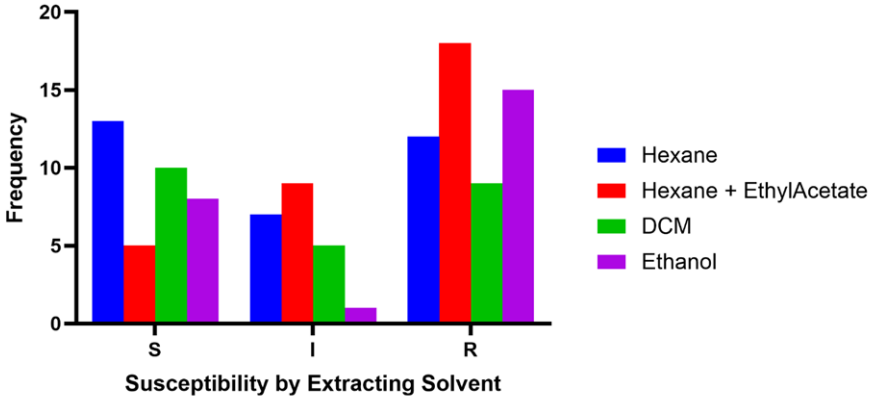

Figure 2. Susceptibility profile of the extracts according to extracting solvent (S, Susceptible; I, Intermediate Susceptible; R, Resistant)

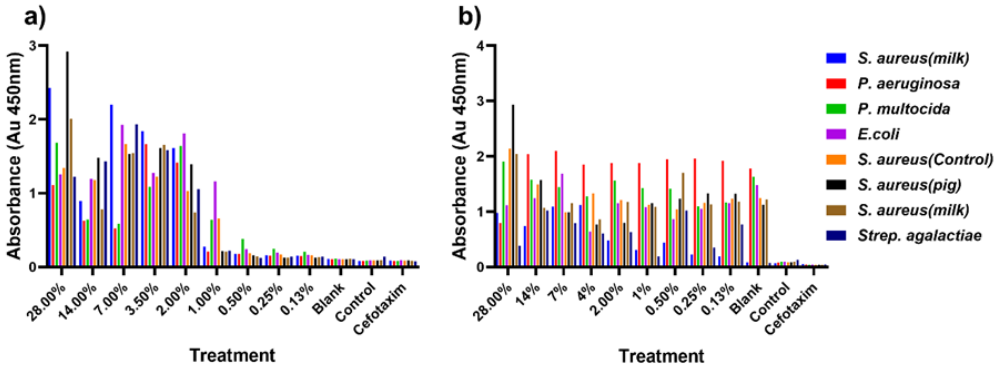

Figure 3. *C. molle* Ethanol Extracts absorbance readings against test organisms at 28% to 0.13% Serial Dilutions (a) Pre-Incubation & (b) Post- Incubation