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Analysis of the correlation between phytochemical content and wound-healing potential of *Lantana camara* ethyl acetate and methanol extracts

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ABSTRACT

Background & Aim: *Lantana camara*, regarded as an invasive plant, is found in tropical climates worldwide. Several studies have validated its antiulcer activity which is one of the ethnomedicinal uses of *L. camara*. This study seeks to find the correlation between the phytochemical content and the wound-healing potential of *L. camara* extracts.

Experimental: The study was conducted using spectrophotometric analysis and selected in vitro bioactivity assays; 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT), inhibitory effect on nitric oxide production in lipopolysaccharide-activated RAW 264.7 macrophages, and 96-well plate micro-broth dilution method assay, of methanol flower (MLF), berry (MLB), leaf (MLL) and ethyl acetate flower (ELF), berry (ELB), leaf (ELL) extracts for cytotoxicity, anti-inflammatory and antimicrobial activities which support wound-healing capabilities.

Results: The highest total polyphenolic content (TPC) was recorded in MLF and ELF extracts (34.59±3.01; 29.50±2.11, mg GAEQ/100 mg). MLF and MLB retained the highest terpenoid concentration (20.74±2.34; 20.51±1.86 mg LIN EQ/100 mg). ELB exhibited anti-inflammatory activity at 200 µg/mL. MLF and ELB were nontoxic to Vero cells, while MLL and MLB caused < 20 % cell death at all concentrations investigated. All ethyl acetate extracts exhibited potent antimicrobial activities against *Streptococcus pyogenes* with both ELL and ELF extracts demonstrating inhibition with MIC ≤ 0.125 mg/mL. The results showed MLF which retained the highest TPC was nontoxic whereas ELL, with relatively high alkaloid content, was the most cytotoxic extract that exhibited growth inhibition to *S. pyogenes* at a significantly low MIC. Thus, the phytochemical content of extracts is positively associated with bioactivity. The nontoxic, anti-inflammatory, and antimicrobial activities support the wound-healing potentials of the extracts.

Recommended applications/industries: The high phenolic content and microbial growth inhibition exhibited could be leveraged to develop wound-healing tinctures or isolate bioactive compounds that could be developed as wound-healing agents.

1. Introduction

Lantana camara L. *(Verbenaceae)* plant extracts have been found effective for treating various diseases traditionally field (Sathish *et al.*, 2011). There is a claim that in Asian countries *L. camara* leaf extracts are used for the treatment of cuts and ulcers (Misha Arpana, 2015; Dubey and Padhy, 2013). Africans use *L. camara* leaf extracts to treat malaria, ulcers, and tumors (Kalita *et al.*, 2012; Khan *et al.*, 2016). Additionally, the extracts also serve as therapies for cuts, rheumatisms, catarrhal infections, tetanus, cancer, chickenpox, swelling, viscera, sores, measles, fevers, and colds (Dubey and Padhy, 2013; Khan *et al.*, 2016). *L. camara* extracts are also known to contribute to wound shrinking besides the potent antimicrobial activity exhibited to inhibit pathogenic infections (Akumu *et al.*, 2014). The concentration of phytochemicals in plant extracts determines their effectiveness in ethnomedicinal applications. The literature review presented that the elevated activity of crude extract might be attributed to the presence of many synergistically active compounds (Junio *et al.*, 2011; Kurin *et al.*, 2012). There is a positive linear correlation between wound-healing potential and total phenolic content for aqueous and methanol extracts, thus plant extracts with high phenolic content tend to be significantly effective in the wound-healing process (Terpinc *et al.*, 2012). The therapeutic potential of *L. camara* extracts could be a result of their abundant phytochemical content.

Although wound healing is a phenomenal process that proceeds naturally, factors such as infections and antibiotic resistance, diabetes, metabolic deficiencies, and age contribute to turning a simple cut into a chronic wound or ulcer (Mirrezaei *et al.*, 2020; Tzaneva *et al.*, 2016). Infections caused by gram-positive cocci contribute greatly to the delay in wound-healing as these microorganisms form poly-microbial associations which lead to biofilm formation field (Smith *et al.*, 2016; Desalu *et al.*, 2011). Dermal wounds are often infected by bacteria including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and fungi such as *Candida albicans* (Negut *et al.*, 2018). *S. pyogenes* strains have been reported to be responsible for invasive infection of wounds, as they have been isolated from the pus, blood tissues, and body fluid of cutaneous wounds (Khan *et al.*, 2020). This study aimed to investigate the link between phytochemical concentrations of the methanol and ethyl acetate extracts of *L. camara* and their wound-healing potential.

2. Materials and Methods

2.1. Solvents and reagents

Reagents and solvents used for extraction, spectrophotometric analysis, and biological activities included LiChosolv® solvents (Merck, Germany); standards and reagents (Sigma Aldrich); PBS with and without Ca^{2+} and Mg^{2+} and trypsin-EDTA 3-(4,5-Dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Melphalan (Tocris Bioscience South Africa); RAW 264.7 mouse macrophages (Cellonex South Africa); lipopolysaccharide (LPS), Griess reagent and aminoguanidine, fluconazole, gentamicin sulfate and vancomycin hydrochloride (Sigma-Aldrich St. Louise, MO, USA); Mueller-Hinton (MH) broth and malt extract broth and dimethyl sulfoxide (Merck, USA); and CellTiter-Blue® reagent (Promega).

2.2. Plant material

Fresh *L. camara* biomass was collected by Ms. Elana Storm, Horticulture Department of Nelson Mandela University, at Summerstrand in the Nelson Mandela Municipality, South Africa in December 2021. Plant authentication was performed by Mr Tony Dold, a curator, and taxonomist at Selmar Schonland Herbarium (GRA) in Makhanda, Eastern Cape, South Africa, a specimen of *L. camara* with voucher number Hlangothi014 (GRA) was deposited.

2.3. Preparation of extraction

The method used for solvent extraction was that described by Agbo *et al.* (2023) and Altemimi *et al.* (2017) with slight modifications based on the study objectives. The biomass of each plant part was extracted sequentially with 100 % ethyl acetate and then methanol (100 %), with biomass to solvent ratio of 1:3 (w/v) using the maceration method for 72 h to obtain crude extracts of leaves, berries, and flowers. Ethyl acetate extracts of the plant parts were labeled ELL, ELB, and ELF whereas methanol ones were MLL, MLB, and MLF. The mass and physical characteristics of each dried extract were then recorded.

2.4. Phytochemical assessment: Quantitative analysis

Spectrophotometric analysis which estimated the extracts' total phytochemical content via the calibration curves of selected standards was conducted for each extract.

2.4.1. Total phenolic content

The total phenolic content of each extract was assessed using the single electron transfer mechanism of the Folin-Ciocalteau reagent as described by Agbo *et al*. (2023) and Kamboj *et al.* (2015), with slight modifications, and expressed in milligram gallic acid equivalent per 100 milligrams of extract (mg GAEQ/100 mg). The standard gallic acid (GA) was used to generate the calibration curve with a linear regression equation;

$$y = 0.0214x + 0.0024;\ R^2 = 0.9832 \quad (1)$$

The absorbances of the standard and the extracts were measured at 760 nm and recorded against reagent blank- methanol.

2.4.2. Total flavonoid content

The total flavonoid content of each extract was estimated using the aluminium-chloride colorimetric assay as described by Agbo *et al.* (2023), with slight modification, and as expressed in milligram quercetin equivalent per 100 milligrams of extract (mg GAEQ/100 mg). Quercetin reference standard was used to generate the calibration curve with a linear regression equation;

$$y = 0.0551x+0.0004;\ R^2=0.9928 \quad (2)$$

The absorbances of the standard and the extracts were measured against the blank at 510 nm using a UV-visible spectrophotometer.

2.4.3. Total terpenoids content

The method for total terpenoid content evaluation was adopted from Agbo *et al.* (2023), estimated and expressed in milligram linalool equivalent per 100 milligrams of extract (mg LINEQ/100 mg). The TTC of the extracts was estimated using a linalool calibration curve with a regression equation;

$$y = 0.0204x+0.001;\ R^2=0.9731 \quad (3)$$

The absorbances of the standard and extracts were measured against a blank at 538 nm.

2.4.4. Total alkaloid content

The total alkaloid content which was estimated and expressed in milligram atropine equivalent per 100 mg of extract (mg ATPEQ/100 mg), was assessed using the bromocresol green (BCG) method with atropine standard as described by Agbo *et al.* (2023) and Shamsa *et al.* (2007), with minor modifications. Atropine was used as the reference standard to generate the calibration curve with a linear regression equation;

$$y = 0.0551x + 0.0004;\ R^2 = 0.992 \quad (4)$$

The absorbances of the yellow BCG- atropine complex and that of the extracts in chloroform were measured at 470 nm against chloroform blank using Shimadzu 3100 UV-VIS spectrophotometer.

2.5. Cytotoxicity testing

Cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

Vero cells (Cellonex, RSA) were cultured in DMEM low glucose cell culture medium supplemented with 10 % FBS. The cells were seeded at a density of 5000 cells/100 µL/well and incubated overnight at 37 °C for attachment. Melphalan at 25, 50, and 100 µM was the positive control. After 48 h exposure of cells to the extracts, the medium was removed. MTT diluted in complete medium to a final concentration of 0.5 mg/mL was added to wells using 100 µL aliquots. After incubation for 3 h at 37 °C, the medium was aspirated, and the purple insoluble formazan product was dissolved by the addition of 100 µL DMSO. The absorbance was measured spectrophotometrically at 540 nm using a BioTek®Power Wave XS spectrophotometer.

2.6. Anti-inflammatory activity

The anti-inflammatory and viability assessments were conducted using the method described by Rampa *et al.* (2022), with aminoguanidine (AG) as the positive control. RAW 264.7 cells (Cellonex, RSA) were seeded into 96-well plates at a density of 1×10^5 cells per well and allowed to attach overnight. The extracts (diluted in DMEM complete medium) were added to give final concentrations of 50, 100, and 200 µg/mL

(50 µL per well). To assess the anti-inflammatory activity, 50 µL of LPS (final concentration of 500 ng/mL) containing medium was added to the corresponding wells. Cells were incubated for a further 24 h. To quantify nitric oxide (NO) production, 50 µL of the spent culture medium was transferred to a new 96-well plate, and 50 µL Griess reagent was added. Absorbance was measured at 540nm and the results expressed relative to the appropriate untreated control. To confirm the absence of toxicity as a contributory factor, cell viability was assessed using MTT.

2.7. Antimicrobial activity testing

The microorganisms used were clinical strains obtained from the NHLS in Port Elizabeth. *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were grown in Mueller-Hinton (MH) broth (Merck, USA) and *C. albicans* was grown in malt extract broth at 37 °C. *Streptococcus pyogenes* was grown in brain-heart infusion broth. One microorganism colony, from an overnight streak plate, was inoculated in 10 mL broth and allowed to grow for 16 h (log growth phase) at 37 °C. Gentamicin sulfate and vancomycin hydrochloride were used as positive controls against gram-negative and gram-positive bacteria, respectively. Fluconazole was used as positive control against *C. albicans*. Antibiotics were dissolved in dH_2O at stock concentrations of 2 mg/mL and filter sterilized (0.2 µM filter). Fluconazole was dissolved in DMSO at stock concentration of 5 mg/mL. Working concentrations were prepared in broth, depending on the antibiotics' MIC values. Antibiotic concentrations used based on bacteria/yeast were as follows: Bacteria: 0.064–0.005 mg/mL and *C. albicans*: 1-0.0078 mg/mL (fluconazole). MH broth/malt extract/brain-heart infusion broth (50 µL) was added to all test wells (i.e., plant extracts and antibiotics), except for the highest plant extract and antibiotic concentration wells to which 100 µL of the working concentrations were added. Serial dilutions were prepared for the plant extracts (2 mg/mL-125 µg/mL) and antibiotics (vancomycin/gentamicin: from 64 to 0.25 µg/mL; fluconazole: 1000-7.81 µg/mL). The cultures were assessed and adjusted to a 0.5 McFarland standard (absorbance at 600 nm = 0.08-0.1; equivalent to ~1.5×10^8 cells/mL) and 50 µL was added to each test well. MIC values were determined through visual inspection of the plate and reported as the lowest concentration (Golparvar *et al.*, 2018).

2.8. Statistical analysis

Each experiment was conducted in quadruplicate. The two-tailed Student t-test and a 2-way analysis of variance (ANOVA) were used to compare data and $P \leq 0.05$ was deemed statistically significant.

3. Results and discussion

The physical appearances and mass yields of extracts of the various plant parts are summarised in Table 1.

Table 1. Yield and physical characteristics of ethyl acetate and methanol extracts.

Solvent	Plant part	The physical appearance of syrups	Starting mass [g]	Quantity [g/20 g] dry biomass
Ethyl acetate	Flowers	Dark green	20.10	2.5
	Berries	Oily-yellowish	40.32	8.9
	Leaves	Dark green	50.46	6.3
Methanol	Flowers	Green	20.10	6.0
	Berries	Oily-greenish	40.32	13.0
	Leaves	Dark green	50.46	14.0

The results showed that methanol was a more suitable solvent for extraction than ethyl acetate. Large quantities of extracts were obtained with methanol whereas the ethyl acetate extracts were about 50 % of the methanol extracts. The berry extract of both polar and nonpolar solvents was oily and this may influence their analysis with polar solvent.

3.1. Quantitative analysis

The results of quantitative analysis showing the phytochemical content of each plant part are summarized in Table 2.

Table 2. Total, phenolic, flavonoids terpenoids, and alkaloids contents.

Solvent	Extract	Total phenol content [mg GAEQ/100 mg]	Total Flavonoid content [mg QEQ/ 100 mg]	Total terpenoids content [mg LIN EQ/100 mg]	Total alkaloids content [mg ATPEQ/ 100 mg]
Methanol	MLB	14.05±4.04	12.45±1.87	**20.51±1.86**	2.05±0.18
	MLL	18.58±1.87	**17.69±2.16**	15.97±1.19	2.60±1.10
	MLF	**34.59±3.01**	**20.41±2.69**	**20.74±2.34**	1.87±1.54
Ethyl acetate	ELB	12.28±3.81	3.75±1.97	10.34±2.52	1.05±0.63
	ELL	17.16±3.20	4.67±1.33	5.76±2.05	1.83±0.61
	ELF	**29.50±2.11**	7.75±1.71	15.63±2.68	1.37±1.44

Values are expressed as mean ± SD of quadruplicate determinations.

A general overview of the results showed that the photochemical methanol extracts of all plant parts were higher than those of ethyl acetate ones. The flower extracts of both solvents retained high concentrations of the major phytochemicals evaluated. The berry extracts on the other hand retained low concentrations except for significantly high terpenoid content.

3.1.1. Total phenolic content

The analysis of the total phenolic content (TPC) of the extracts was based on the fact that in an alkaline medium, phenols reduce the mixture of phosphotungstic and phosphomolybdic acid present in the Folin-Ciocalteu reagent to a blue-colored tungsten and molybdenum oxide chromophore which is proportional to the concentrations of phenolic compounds present in the extracts (Kamboj *et al.*, 2015, Agbo *et al.*, 2015). The results showed that the TPC of the MLF (34.59±3.01 mg GAEQ/100 mg), was the highest recorded, while the lowest was that of the ELB (12.28±3.81 mg GAEQ/100 mg). The results were compared with the findings of Anwar *et al.* (2013), which showed TPC (21.40 and 18.43 g GAE/100 g DW) in *L. camara* ethanol flower and leaf extracts respectively. The wound-healing potential of medicinal plants has been attributed to the presence of phytochemicals including alkaloids, polyphenolic compounds, and terpenoids (Shedoeva *et al.*, 2019). Polyphenolic compounds aid the process of tissue remodeling, and cell signaling, and act as proangiogenic agents during wound healing (Thangapazham *et al.*, 2016, do Carmo *et al.*, 2018, Shimizu *et al.*, 2017). They also serve as a defense to the skin through processes such as quenching of free radicals and reduction of inflammation (Kochman *et al.*, 2021). Elevated concentrations of these chemicals are therefore beneficial to the wound-healing process.

3.1.2. Total flavonoid content

The total flavonoid content (TFC) of the extracts was measured based on the formation of a yellow flavonoid-Al^{3+} complex whose intensity is proportional to the concentration of flavonoids in the extract. Aluminium (Al^{3+}) reacts with OH groups of flavonoids from the extract to form a stable flavonoid-Al^{3+} complex (Agbo *et al.*, 2015). The results showed that MLF retained the highest TFC, (20.41±2.69 mg QEQ/100 mg), while the ELB retained the least TFC (3.75±1.97 mg QEQ/100 mg). The TFC of flower extracts was highest in both solvents and least in the berry extracts. The results were compared with the findings of Anwar *et al.* (2013) which estimated TFC at 13.8 mg catechin equivalent/mg dry weight of methanol leaf extract. Flavonoids have efficacy for wound healing as they are distinctly involved in the inhibition of fibroblast proliferation, and prevent oxidative damage besides serving as antibacterial agents (Zulkefli *et al.*, 2023). A high total flavonoid content could therefore contribute to the wound-healing potential of the extracts.

3.1.3. Total terpenoid content

The assay for assessing the total terpenoid content (TTC) in extracts was based on the reaction of linalool standard reagent with concentrated sulfuric acid to form a brick red precipitate which is partially soluble in reaction mixture solution and chloroform but fully in methanol (do Carmo *et al.*, 2018). The absorbance of this brick red precipitate is therefore proportional to the concentration of the linalool. The results yielded that the TTC of MLF and MLB (20.74±2.34 and 20.51±1.86 LIN EQ/100mg) were the highest respectively, while the least TTC was that of ELL extract (5.76±2.05 mg LIN EQ/100 mg). Previous studies on the total terpenoid content for *L. camara* extracts were not found during this study.

3.1.4. Total alkaloid content

The assessment of total alkaloid content (TAC) was based on the formation of a chromophore of yellow alkaloid-bromocresol green (BCG) complex and the absorbance of this complex is proportional to the concentration of alkaloids present (Shamsa *et al.*, 2007). The results are outlined in Table 2.

The results showed that the TAC of the MLF (2.60±1.10 mg ATPEQ/100 mg) was the highest while that of the ELB (1.05±0.63 mg ATPEQ/100 mg) was the least. This could be due to the oily nature of the berry extracts. The overall low TAC recorded in this study could be due to the basic nature of which might interfere with the concentration of alkaloids in the extracts.

3.2. In vitro bioassays of *Lantana camara* extracts

3.2.1. Cytotoxicity

Six samples consisting of three ethyl acetate and three methanol extracts of *L. camara* (ELB, ELF, ELL, MLB, MLF, MLL), were screened against Vero kidney cells using an MTT assay, and the results are summarised in Figure 1A and B.

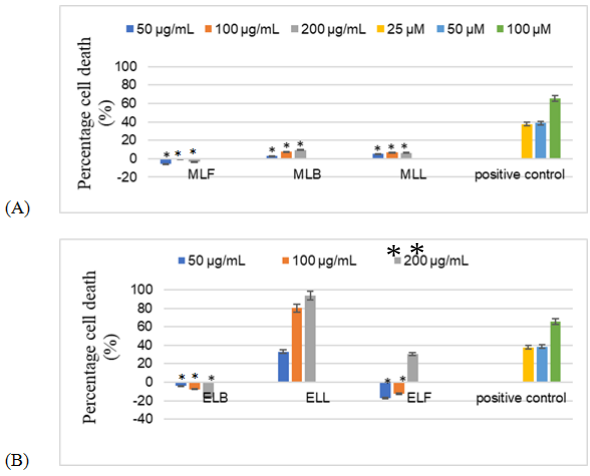

Figure 1. Cytotoxicity of *L. camara* extracts on Vero African green monkey kidney cells; (A): MeOH [MLF, MLB, MLL] (B): EtOAc [ELF, ELB, ELL]; Melphalan was used as a positive control at 25, 50, and 100 µM; *Indicates statistical significance compared to the control; P< 0.05 using two-tailed Student's *t*-test.

The percentage of cell death ranged between 37.64±1.90 and 65.50±0.93% at a concentration ranging between 50 and 200 µg/mL.

MLF and ELB were nontoxic while MLL and MLB were found to cause < 20 % cell death at all concentrations under investigation. The highest total phenolic content (TPC) of MLF could explain its nontoxicity since phenols reduce oxidative stress which is the key route to cytotoxicity (Osman *et al.*, 2020). ELL was the most cytotoxic extract to the Vero cell line, inducing cell death at a percentage ranging between 33.17±12.87 and 93.45±0.81 % at a concentration range of 50 to 200 µg/mL. ELF was slightly toxic to the Vero cell line with cell death percentages of -12.19±3.07 and 30.32±5.36 % at the same concentrations. The toxicity difference could be linked to the TPC of the two extracts while the TPC of ELF was 29.50±2.11 mg GAEQ/100 mg, and that of ELL was 17.16±3.20 mg GAEQ/100 mg. Generally, medicinal plants' phytochemical content positively correlates with their bioactivity and the results of this study are a confirmation of that although the activity of ELB appeared as an outlier. The results showed that these extracts may cause stimulation of cell proliferation and present an advantageous effect in the wound healing and skin treatment process (Ghuman *et al.*, 2016).

3.2.2. Anti-inflammatory activity

The evaluation of the inhibition of NO production in the LPS-stimulated RAW 264.7 macrophages was used to estimate the anti-inflammatory activity of the extracts while the cell viability assessment test was conducted using the MTT assay. The nitrite concentration was measured using the Griess reagent after 24 h incubation with the samples and 200 ng LPS to activate the inflammatory response. The MTT values were used to normalize the nitrite levels (Figure 2). Nitrite levels were excluded from the graph as shown in the figure below, where cell viability was <50 %.

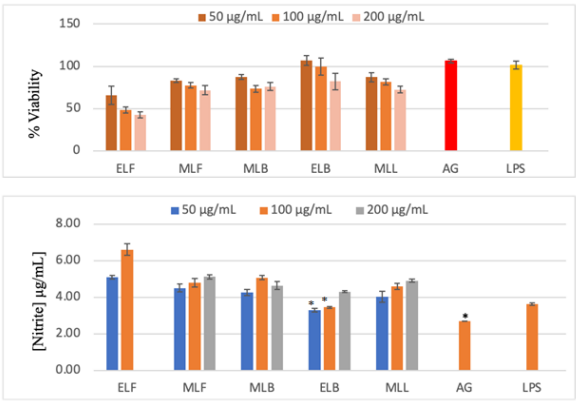

Figure 2. Cytotoxicity (top) and anti-inflammatory activity (bottom) of *Lantana camara* extracts on RAW 264.7 macrophages. MTT values were used to normalize nitrite levels in the bottom figure. MLL = methanol *Lantana* leaf; MLF = methanol *Lantana* flower; MLB = methanol *Lantana* berry; ELL = ethyl acetate *Lantana* leaf; ELF = ethyl acetate *Lantana* flower; ELB = ethyl acetate *Lantana* berry. Data points represent the mean ± SD of 4 replicate wells. *P<0.05; **P<0.01: significantly lower than LPS-activated control cells (LPS).

The majority of the extracts (ELF, MLF, MLB, MLL) showed a negative cell viability effect on the macrophages at concentrations ranging from 50-200 µg/mL. ELB however, exhibited a significant anti-inflammatory activity, inhibiting NO• radical generation in the LPS-stimulated RAW 246.7 macrophages at concentrations ranging from 50 and 100 µg/mL without affecting cell viability.

Previous research findings showed that *L. camara* flower extract could be a potent anti-inflammatory agent when used to synthesize zinc oxide nanoparticles. The *L. camara* zinc oxide nanoparticles served as an anti-inflammatory agent that inhibited the production of phospholipase A2 enzyme with a MIC value of 41 µg/mL (Surendra *et al.*, 2021). *L. trifolia* ethanol leaf extract exhibited a powerful reduction in the paw edema induced by carrageenan, serotonin, or histamine with inhibition values ranging from 72.9 to 90.0 % in the model of rat paw edema (Silva *et al.*, 2005). The ethyl acetate berry extracts exhibited anti-inflammatory activity at a low concentration, indicating that their application could help control the inflammatory stage of the wound-healing process. The phytochemical content of ELB could not be related to this activity, since low concentrations were estimated in extracts.

3.2.3. Antimicrobial activity testing

The micro-broth dilution susceptibility method in a 96-well microliter plate was used in the evaluation of the antimicrobial activity of methanol and ethyl acetate extracts of three plant parts of *L. camara* (berries, flowers, and leaves) against wound infection microorganisms (*E. coli*, *P. aeruginosa*, *S. aureus*, *S. pyogenes*, and *C. albicans*). The minimum inhibitory concentration (MIC) is a qualitative assessment estimated by a color change from blue to pink/purple color due to the reduction of the CellTiter-Blue® metabolic dye through viable bacteria and the data generated is summarized in Table 3.

Table 3. MIC values of extracts screened against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*.

Sample	MIC (mg/mL)				
	S. aureus	***S. pyogenes***	***E. coli***	***P. aeruginosa***	***C. albicans***
MLL	> 2	2	> 2	> 2	> 2
MLF	> 2	> 2	> 2	> 2	> 2
MLB	> 2	> 2	> 2	> 2	> 2
ELL	> 2	≤ 0.125	> 2	> 2	> 2
ELF	> 2	≤ 0.125	> 2	> 2	> 2
ELB	> 2	0.5	> 2	> 2	> 2

MLL = methanol *Lantana* leaf extract; MLF= methanol *Lantana* flower extract; MLB = methanol *Lantana* berry extract; ELL = ethyl acetate *Lantana* leaf extract; ELF = ethyl acetate *Lantana* flower extract; ELB = ethyl acetate *Lantana* berry extract.

The ethyl acetate extracts of all the plant parts exhibited growth inhibition against *S. pyogenes* at a promising MIC ranging between ≤ 0.125–0.5 mg/mL. This could be due to their low phenolic content which is known to be a route in inducing cytotoxicity. Both ELL and ELF demonstrated strong activity with inhibition at MIC ≤ 0.125 mg/mL against *S. pyogenes* while there was growth inhibition at MIC 0.5 mg/mL and 2 mg/mL exhibited by ELB and MLL respectively.

A 2-way analysis of variance (ANOVA) was used to determine statistically the effect of the crude samples on the MIC values of the *S. pyogenes and P. aeruginosa* microorganisms and the results is shown in Table 4.

Table 4. Statistical analysis of MIC values using ANOVA.

Source of Variation	SS	DF	MS	F-value	P-value	F crit
Rows	15.39781	5	3.079562	1.06929	0.471593	5.050329
Columns	2.59284	1	2.59284	0.90029	0.386275	6.607891
Error	14.40003	5	2.880007			
Total	32.39068	11				

SS = sum of squares; DF= degrees of freedom; MS = mean squares .

There was no significant difference statistically in the means of the MIC values due to the type of crude extract used and the organisms tested. The results also showed that statistically, the crude extracts had a similar effect on the MIC values, $F = 1.07 < 5.05 = F\text{-}crit$ and the influence of the type of microorganism on the MIC values was also not statistically significant, F (0.91) = 6.61, $P < 0.05$. It can be concluded that there is no significant difference statistically in the effect of the six crude extracts on the type of microorganism as well as on the MIC values obtained.

The results were compared with the findings of Alfaray *et al.* (2020), which showed that the ethanol flower extracts inhibit the growth of *S. pyogenes* at a MIC of 250 mg/mL. ELL which retained the least total phenolic content (17.16±3.20mg GAEQ/100 mg) and a significant total alkaloid content (1.83±0.61 mg ATPEQ/ 100 mg) compared to other plant parts, exhibited the highest toxicity and this could explain the lowest MIC at which it inhibited the growth of *S. pyogenes.* The high total phenolic, terpenoid, and flavonoid contents of ELFwere positively associated with its antibacterial activity.

4. Conclusion

Findings of the study showed that both methanol and ethyl acetate flower extracts of *L. camara* retained high polyphenolic content as well as terpenoid content, MLF was nontoxic at all concentrations while ELF was nontoxic at low concentrations. The low MIC at which ELL inhibited the growth of *S. pyogenes* could be attributed to its high toxicity due to low total phenolic and relatively high total alkaloid contents whereas the growth inhibition of ELF was associated with the relatively high terpenoid and flavonoid contents. ELB exhibited a moderate anti-inflammatory activity because it was nontoxic however, this could not be linked with any of the phytochemical contents estimated. Ethyl acetate extracts were nontoxic and found to exhibit potent antimicrobial and anti-inflammatory activities. Largely, there was a positive association between phytochemical content and the bioactivity of plant extracts.

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6. References

Agbo, I.A., Hlangothi, B., Didloff, J., Hattingh, A.C., Venables, L., Govender, S. and De Venter, M. 2023. Comparative evaluation of the phytochemical contents, antioxidant and some biological activities of *Khaya grandifoliola* methanol and ethyl acetate stem bark, root, and leaf extracts. *Tropical Journal of Natural Product Research*, 7: 2829-2836.

Agbo, M.O., Uzor, P.F., Nneji, U.N.A., Odurukwe, C.U.E., Ogbatue, U.B. and Mbaoji, E.C. 2015. Antioxidant, total phenolic, and flavonoid content of selected Nigerian medicinal plants. *Dhaka University Journal of Pharmaceutical Sciences*, 14: 35-41.

Akumu, E.O., Swamy, A.T., Mutuku, C.N., Obey, J.K. and Miyogo, E. 2014. Pharmacological evaluation of *Lantana camara* leaf smoke. *International Journal of Pharmacy and Biological Sciences*, 4: 28-34.

Altemimi, A., Lakhsassi, N., Baharlouei, A., Watson, D.G. and Lightfoot, D.A. 2017.Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6: 1-23.

Desalu, O.O., Salawu, F.K., Jimoh, A.K., Adekoya, A.O., Busari, O.A. and Olokoba, A.B. 2011. Diabetic foot care: Self-reported knowledge and practice

among patients attending three tertiary hospitals in Nigeria. *Ghana Medical Journal*, 45: 60-65.

do Carmo, M.A.V., Pressete, C.G., Marques, M.J., Granato, D. and Azevedo, L. 2018. Polyphenols as potential antiproliferative agents: Scientific trends. *Current Opinion in Food Science*, 24: 26-35.

Dubey, D., Padhy, R.N. 2013. Antibacterial activity of *Lantana camara* L. against multidrug-resistant pathogens from ICU patients of a teaching hospital. *Journal of Herbal Medicine*, 3: 65-75.

Ghuman, S., Ncube, B., Finnie, J.F., McGaw, L.J., Coopoosamy, R.M. and Van Staden, J. 2016. Antimicrobial activity, phenolic content, and cytotoxicity of medicinal plant extracts used for treating dermatological diseases and wound-healing in KwaZulu-Natal, South Africa. *Frontiers in Pharmacology*, 7: 1-9.

Golparvar, A.R., Gheisari, M.M., Hadipanah, A. and Khorrami, M. 2018. Antibacterial, antifungal properties and chemical composition of essential oils of *Satureja hortensis* L. and *Satureja khuzestanica* Jamzad. *Journal of Medicinal Herbs*, 8: 243-249.

Heping, C., Kandan, S. 2020. Regulation of cell viability and anti-inflammatory *Tristetraprolin* family gene expression in mouse macrophages by cotton seed extracts. *Scientific Reports (Nature Publisher Group)*, 10: 1-11.

Junio, H.A., Sy-Cordero, A.A., Ettefagh, K.A., Burns, J.T., Micko, K.T., Graf, T.N., Richter, S.J., Cannon, R.E., Oberlies, N.H. and Cech, N.B. 2011. Synergy-directed fractionation of botanical medicines: A case study with golden seal (*Hydrastis canadensis*). *Journal of Natural Products*, 74: 1621-1629.

Kalita, S., Kumar, G., Karthik, L. and Rao, K.V.B. 2012. A Review on medicinal properties of *Lantana camara* Linn. *Research Journal of Pharmacy and Technology*, 5: 711-715.

Kamboj, A., Gupta, R., Rana, A. and Kaur, R. 2015. Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from extracts of *Terminalia bellerica*. *European Journal of Biomedical and Pharmaceutical Sciences*, 2: 201-215.

Khan, M., Mahmood, A. and Alkhathlan, H.Z. 2016. Characterization of leaves and flowers volatile constituents of *Lantana camara* growing in the Central Region of Saudi Arabia. *Arabian Journal of Chemistry*, 9: 764-774.

Khan, R.M.A., Anwar, A., Pirzada, Z.A. 2020. *Streptococcus pyogenes* strains associated with invasive and non-invasive infections present possible links with emm types and superantigens. *Iranian Journal of Basic Medical Sciences*, 2020: 133-139.

Kochman, J., Jakubczyk, K., Antoniewicz, J., Mruk, H. and Janda, K. 2021. Health benefits and chemical composition of matcha green tea: A review. *Molecules*, 26: 1-11.

Kurin, E., Atanasov, A.G., Donath, O., Heiss, E.H., Dirsch, V.M. and Nagy, M. 2012. Synergy study of the inhibitory potential of red wine polyphenols on vascular smooth muscle cell proliferation. *Planta Medica*, 78: 772-778.

Mirrezaei, N., Yazdian-Robati, R., Oroojalian, F., Sahebkar, A. and Hashemi, M. 2020. Recent developments in nano-drug delivery systems loaded by phytochemicals for wound healing. *Mini-Reviews in Medicinal Chemistry*, 20: 1867-1878

Misha, A. 2015. Allelopathic properties of *Lantana camara*. *International Research Journal of Basic and Clinical Studies*, 3: 13-28.

Negut, I., Grumezescu, V., Grumezescu, A.M. 2018. Treatment strategies for infected wounds. *Molecules*, 23: 1-23.

Njayou, F.N., Aboudi, E.C.E., Tandjang, M.K., Tchana, A.K., Ngadjui, B.T. and Moundipa, P.F. 2013. Hepatoprotective and antioxidant activities of stem bark extract of *Khaya grandifoliola* (Welw) CDC and *Entada africana* Guill. et Perr. *Journal of Natural Products*, 6: 73-80.

Osman, M.A., Mahmoud, G.I. and Shoman, S.S. 2020. Correlation between total phenol content, antioxidant power, and cytotoxicity. *Biointerface Research in Applied Chemistry*, 11: 10640-10653.

Rampa, K.M., Van De Venter, M., Koekemoer, T.C., Swanepoel, B., Venables, L., Hattingh, A.C., Viljoen, A.M. and Kamatou, G.P., 2022. Exploring four South African *Croton* species for potential anti-inflammatory properties: *In vitro* activity and toxicity risk assessment. *Journal of Ethnopharmacology*, 282: 1-11.

Sathish, R., Vyawahare, B., Natarajan, K. 2011. The antiulcerogenic activity of *Lantana camara* leaves on gastric and duodenal ulcers in experimental rats. *Journal of Ethnopharmacology*, 134: 195-197.

Shamsa, F., Monsef, H.R., Ghamooghi, R. and VerdianRizi, M.R. 2007. Spectrophotometric determination of total alkaloids in *Peganum harmala*

L. using bromocresol green. *Research Journal of Phytochemistry* 1: 79-82.

Shedoeva, A., Leavesley, D., Upton, Z. and Fan, C. 2019. Wound-healing and the use of medicinal plants. *Evidence-Based Complementary and Alternative Medicine*, 2019:1-35.

Shimizu, M. 2017. Multifunction of dietary polyphenols in the regulation of intestinal inflammation. *Journal of Food and Drug Analysis*, 25: 93-99.

Silva, G.N., Martins, F.R., Matheus, M.E., Leitão, S.G. and Fernandes, P.D. 2005. Investigation of anti-inflammatory and antinociceptive activities of *Lantana trifolia*. *Journal of Ethnopharmacology*, 100: 254-259.

Smith, K., Collier, A., Townsend, E.M., O'Donnell, L.E., Bal, A.M., Butcher, J., Mackay, W.G., Ramage, G. and Williams, C. 2016. One step closer to understanding the role of bacteria in diabetic foot ulcers: Characterising the microbiome of ulcers. *BMC Microbiology*, 16: 1-12.

Surendra, B.S., Mallikarjunaswamy, C., Pramila, S. and Rekha, N.D. 2021. Bio-mediated synthesis of ZnO nanoparticles using *Lantana Camara* flower extract: Its characterizations, photocatalytic, electrochemical and anti-inflammatory applications. *Environmental Nanotechnology, Monitoring & Management*,15: 1-10.

Terpinc, P., Čeh, B., Ulrih, N.P. and Abramovič, H. 2012. Studies of the correlation between antioxidant properties and the total phenolic content of different oil cake extracts. *Industrial Crops and Products*, 39: 210-217.

Thangapazham, R.L., Sharad, S., Maheshwari, R.K. 2016. Phytochemicals in wound healing. *Advances in Wound Care*, 5: 230-241.

Tzaneva, V., Mladenova, I., Todorova, G. and Petkov, D. 2016. Antibiotic treatment and resistance in chronic wounds of vascular origin. *Clujul Medical*, 89: 365-370.

Zulkefli, N., Che Zahari, C.N.M., Sayuti, N.H., Kamarudin, A.A., Saad, N., Hamezah, H.S., Bunawan, H., Baharum, S.N., Mediani, A., Ahmed, Q.U. and Ismail, A.F.H., 2023. Flavonoids as potential wound-healing molecules: Emphasis on pathways perspective. *International Journal of Molecular* 24:1-29.