Bulletin of Biological and Allied Sciences Research
***ISSN:* 2521-0092**
www.bbasr.org
DOI: https://doi.org/10.54112/bbasr.v2023i1.33
*Bull. Biol. All. Sci. Res., Volume, **8**: 33*

Original Research Article

ANTIMICROBIAL ACTIVITY OF *LANTANA CAMARA* AGAINST *PSEUDOMONAS AERUGINOSA, SERRATIA MARCESCENS AND STAPHYLOCOCCUS AUREUS* TO DEVELOP OINTMENT BASED THERAPY

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(Received, 10th February 2022, Revised 27th February 2023, Published 9th March 2023)

***Abstract*:** *Plant extract (Lantana camara) and earthworm (Pheretima posthuma) extract have been used to treat many illnesses for centuries; they have been reported to suppress the growth of many pathogenic bacteria and are specially used for wound healing. We analyzed the antibacterial activity of the different organic extracts obtained from these species against pathogenic strains of bacteria and compared them to know which is more potent for developing antiseptic ointment. Ethanol and acetone solvents were used for the extraction and analyzed for their antibacterial activity by the disk diffusion and shake flask test. Then we identified the minimum inhibitory concentration of extracts against gram-positive and gram-negative strains. Lantana camara showed the largest growth inhibitory zone against Pseudomonas aeruginosa, followed by Serratia marcescens, and Staphylococcus aureus. MIC for plant extract was 150µg/ml, while earthworm extract inhibited growth at a minimum dose of 200µg/ml. We found that the plant's ethanol extract (L. camara) exhibited robust antimicrobial activity compared to the worm extract. Current investigation reinforces the application of Lantana camara extract to develop ointment to treat wound infections.*

*[Citation: Din, S.Z.U., Fazal, M.O., Ishtiaque, A., Ullah, A. (2023). Comparative analysis of different pathogenic microbe collected from mammalian milk. Bull. Biol. All. Sci. Res. **8**: 33. doi: https://doi.org/10.54112/bbasr.v2023i1.33]*
Keywords: *Lantana camara*, *Pheretima posthuman*, pathogenic bacteria, antibacterial, organic extracts

Introduction

Lantana camara is a flowering plant native to the American tropics but later introduced to other parts of the world. The British brought this as an ornamental plant around 200 years ago in India. Since then, it has been regarded as an invasive plant (Kannan, Shackleton, & Shaanker, 2013). Different parts of plants have been used to treat swellings, eczema, dysentery ulcers, cough, asthma, malaria, rheumatism, fever, and fistula (Deena & Thoppil, 2000). *L. camara* extract has been reported to suppress the growth of many bacteria and fungi and is specially used for wound healing. (Nayak, Raju, & Ramsubhag, 2008). Many bioactive fractions like alkaloids, Flavonoids, Phenols, Tannins, and Terpenoids have been detected in the extract and seem to have a role in antimicrobial activity (Nirmal et al., 2020). Earthworms live in soil which increases soil fertility. This organism has to live side by side with different diversity of bacteria. Some antimicrobial agents may protect the earthworm from harmful bacteria in the soil. Earthworms have been used as medicine since 1340 AD (Bhorgin & Uma, 2014). Its powder contains lysenins, lubricin, and eiseniapore, which have thrombolytic and Antibacterial properties (Cho, Park, Yoon, & Kim, 1998). In this study, we evaluated the antibacterial activity of ethanol and acetone extracts of L. camara and compared them with the antibacterial activity of ethanol and acetone extracts obtained from earthworms' skin (*Pheretima posthuma*). Antibiotic resistance is a serious phenomenon world is facing these days. Developing countries like Pakistan also encounter antibiotic abuse, resulting in many resistance strains. There is a dire need to continuously search for new drugs and treatments to tackle such problems. Another problem is that the drugs may have toxic effects, so natural products are considered the robust approach to treating such wound infections. There is a continuous effort to screen natural resources or organisms to find active compounds which could give us novel active compounds against pathogenic strains. Plants are commonly believed to be rich in such active compounds and more effective than animals. Here we are doing preliminary screening for two organism

extracts for such active compounds and comparing which is more potent for developing ointment for wound healing.

Material and Methods

Plant extracts

Lantana camara plants were collected from the Institute of Microbiology and Molecular Genetics, Punjab University, Lahore, and identified in the lab. Leaves of the plants were washed with tap water until no impurities remained, then dried in the oven. The leaves of plants were ground with pestle mortar, and 10 grams of powder was weighed, put in a flask with 100 ml of ethanol, left soaked for one day, and then stirred on a magnetic stirrer. The mixture was filtered with Whatman grade 42 filter paper. This extract was stored in the refrigerator for further use same procedure was used for acetone extract.

Earthworm extracts

Approximately 200-300 Earthworm were collected from the home backyard at Thokar Niaz Baig Lahore, Pakistan, identified in the lab and soaked in distilled water for 6 hours to remove soil from the gut of earthworms, then kept in an incubator for 24 hours at 55C. After that, earthworms were pounded to make it powder. 10g of powder was put into the flask with 100 ml ethanol, soaked for one day, and stirred on a magnetic stirrer. The mixture was filtered with Whatman 42 filter paper, and this extract was stored in the refrigerator for further use.

Microorganism used

Pure cultures of bacterial strains *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, and *Serratia marscene* were used.

Culture Media and inoculum

The media used for microbial culture was a nutrient medium. Bacterial cultures inoculated were incubated at 37C for 24 hours.

Determination of Antibacterial Activity

The antimicrobial activity of the extracts was determined by the disk diffusion and shake flask test (Rios, Recio, & Villar, 1988). 20 ml of sterile nutrient broth in a 100 ml flask was inoculated by a single colony from a stock culture with a loop and incubated in a shaking incubator at 37C and 110 rpm for 24 hours. After overnight incubation, bacterial culture was diluted in a Nutrient broth of 20 ml for 3 hours at 37 C and 110 rpm. Then one ml of this was serially diluted 3fold to get a target concentration of optical density of 0.1 to 0.3, roughly equal to 1.3 × 10^8 CFU/ml.

Disk diffusion method

200µl of this culture was spread on nutrient agar plates. A well was prepared with a pasture pipette, 200µl of the extract was loaded into the well, and the control well was loaded with the respective solvent. Plates were incubated at 37C for 24 hours. The antibacterial activity was assessed by measuring the inhibition zone. Images were recorded, and the zone of inhibition was measured with a ruler. All the tests were performed in triplicate.

Shake flask test (semi-Quantitative test)

This test was performed to determine growth inhibition. The inoculated medium used was 100X dilution of the 3-hour culture of E. coli. The flasks were then incubated on a shaking incubator at 37C +/- 2C. The absorbance was monitored at 660nm from 0 to 3 hours with 50min intervals, and the final reading was taken at 24 hours. Growth inhibition was accessed based on triplicate results.

Minimum Inhibitory Concentration (MIC)

The powder of each extract was prepared at 10 mg/ml and then diluted to four different concentrations (50, 100, 150, and 200) µg/ml. 100 µl of each strain with a target concentration of roughly 1.3 × 10^8 CFU/ml was added to test dilutions of different extract concentrations except the control test tube, which contained no extract. After 24-hour samples were checked for turbidity, MIC is the lowest concentration of the extract, which inhibits the organism's growth completely. All tests were done in triplicates, and mean values for optical density were visualized with R-Studio

Statistical Software

R Program and excel spreadsheets were used for statistics ANOVA, post-hoc test and visualization of results. A ruler was used for the measurement of zones.

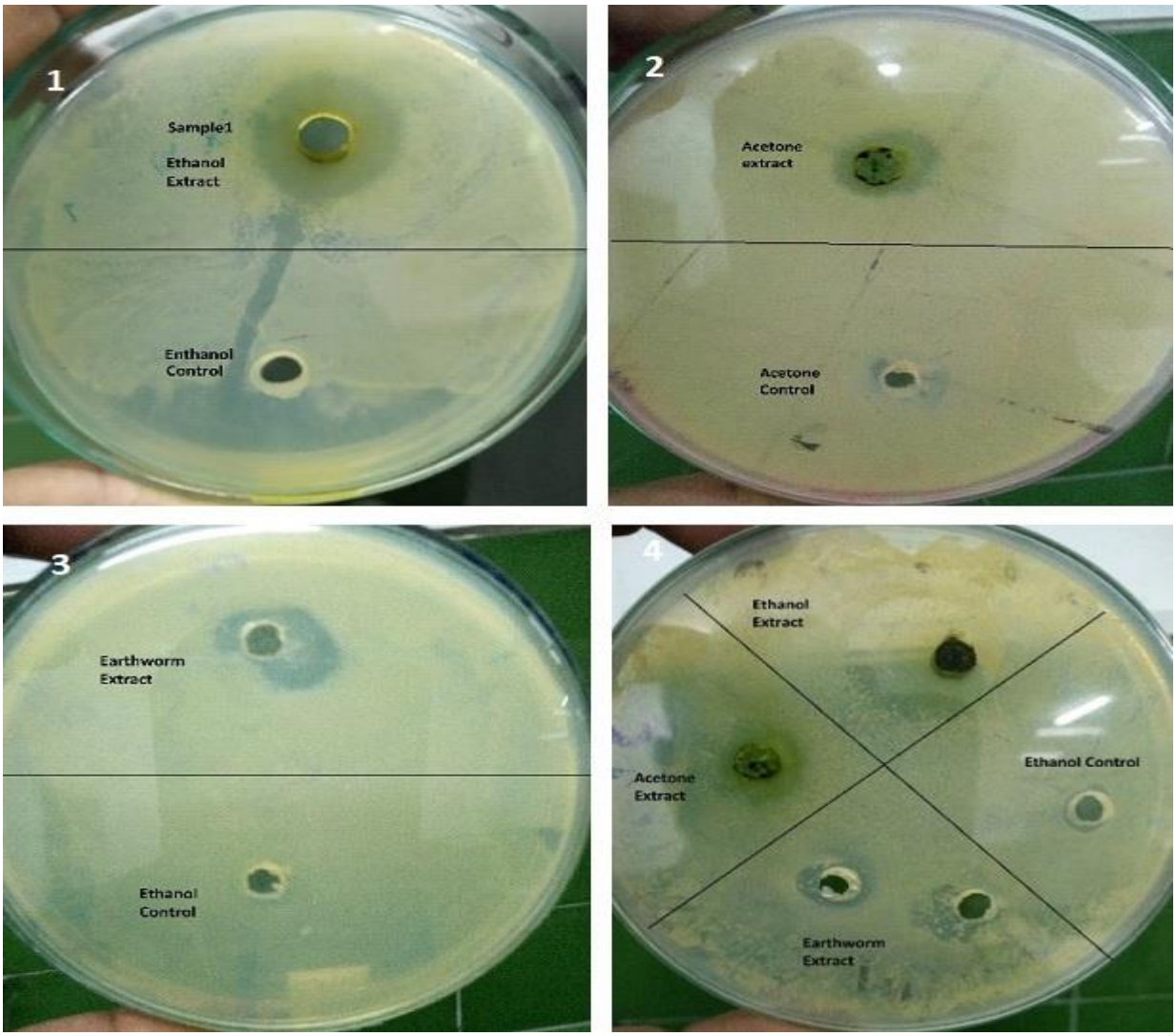
Results and discussion

The present work highlights the use of leaf extracts of the plant (*L. camera)* and earthworm (*Pheretima posthuma*). *L. camara* has been reported to contain almost 29 active fractions like tannins, saponins, steroids, and terpenoids which are potent antimicrobial agents and have been reported to inhibit the growth of bacteria (Nirmal et al., 2020). Similar findings have been reported (Byzov, Khomyakov, Kharin, & Kurakov, 2007), highlighting that the gut fluid of earthworm have some potent antibacterial metabolites of symbiotic bacteria does not lower with partial sterilization of the gut (Bhorgin & Uma, 2014). Earthworm gut has a rich diversity of microbes, but their environment is also very rich in bacterial activity, so it is rational to think there may be some anti-infection agent in the skin of earthworms. Antibacterial activity was first assessed by the disc diffusion method. Extracts were poured into wells on agar plates, and results were noted in table 1. Figure 1 showed a clear zone of inhibition around wells with extract, whereas the control well showed no sign of inhibition. The greatest inhibitory effect was observed against *Pseudomonas aeruginosa* with a zone of inhibition of 9 mm diameter with ethanol plant extract, followed by *Serratia marscene* with a zone of inhibition of 7.5mm diameter.

Table 2 Antimicrobial assessment by disc diffusion method

Samples	Organism	Zone of Inhibition(mm)				
Lantana camara		1	2	3	Mean	St. Dev

Ethanol	*Pseudomonas aeruginosa*	9	8.4	7.8	9.0	±0.6
	Staphylococcus aureus	7.3	6.2	7.4	6.96	±0.6
	E. coli	6.3	6.8	6.9	6.66	±0.32
	S. marcsene	7.5	6.5	7	7	±0.5
Acetone	*S. aureus*	5	4.4	5	5.06	±0.28
	Pseudomonas aeruginosa	5	6.4	6	5.8	±0.72
	E. coli	5	6	5	5.3	±0.57
	S. marscene	5.5	5.8	5	5.43	±0.40
Earthworm (Et.OH.)	*S. aureus*	3.5	3.8	4	3.76	±0.25
	P. aeruginosa	5	5.3	5.5	5.26	±0.25
	E. coli	7	6.5	6	8.2	±0.5
	S. marscene	7.2	7.5	8	7.56	±0.40
Acetone	*S. areus*	4	5	4	4.3	±0.57
	E. coli	5	5	5.3	5.1	±0.17
	P. aeruginosa	4	3.7	3.4	3.7	±0.3
	S. marscene	4	4.7	3	3.9	±0.85



1- Pseudomonas aeruginosa, 2-Staphylococcus aureus, 3-E. coli, 4-Serratia marscene

Figure 1 Antibacterial activity of Extracts showing zone of inhibition against tested bacteria by a disc diffusion method.

In the case of the semi-Quantitative test with nutrient broth, bacterial growth was monitored by measuring the optical density of the medium over time, and reduction with both extracts was measured against *E. coli* as shown in figure 2.

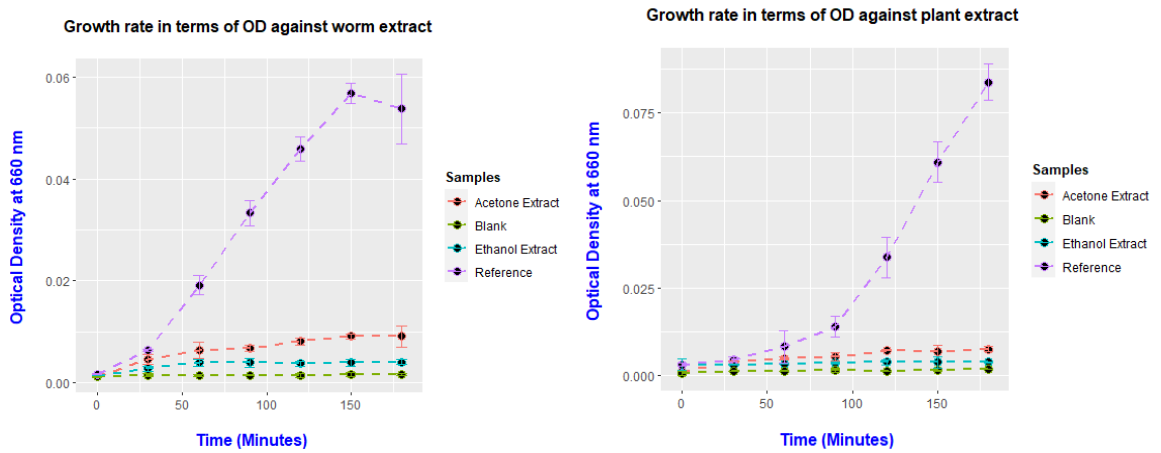


Figure 2 Shows the change in absorbance over time (3 hours) for nutrient broth shake flask tests. Values plotted are mean ± SD, n = (3)

In Figure 2, graphs show the change in absorbance over time. A potent antimicrobial activity was seen against *E. coli*. This method is used to check antibacterial activity within 3 hours. Absorbance measurements are not as accurate as others for determining viable bacteria but can give a rapid reduction estimate. Absorbance measurements (turbidity) are commonly used for minimum inhibitory concentration (MIC) tests. Using the absorbance value, there was a maximum 99% reduction in the growth against plant extract, while an 81% reduction was observed for worm extracts against *E. coli*. The MIC results showed plant extracts with ethanol have an excellent antigrowth inhibitory effect at a dose of 150µg/ml, while acetone extract of the plant showed maximum effect at 200µg/ml. While extract from the worm exhibited good antibacterial inhibitory activity against all strains at a dose of 200µl. Figure 3 shows the effect of the concentration of each extract against bacterial strains.

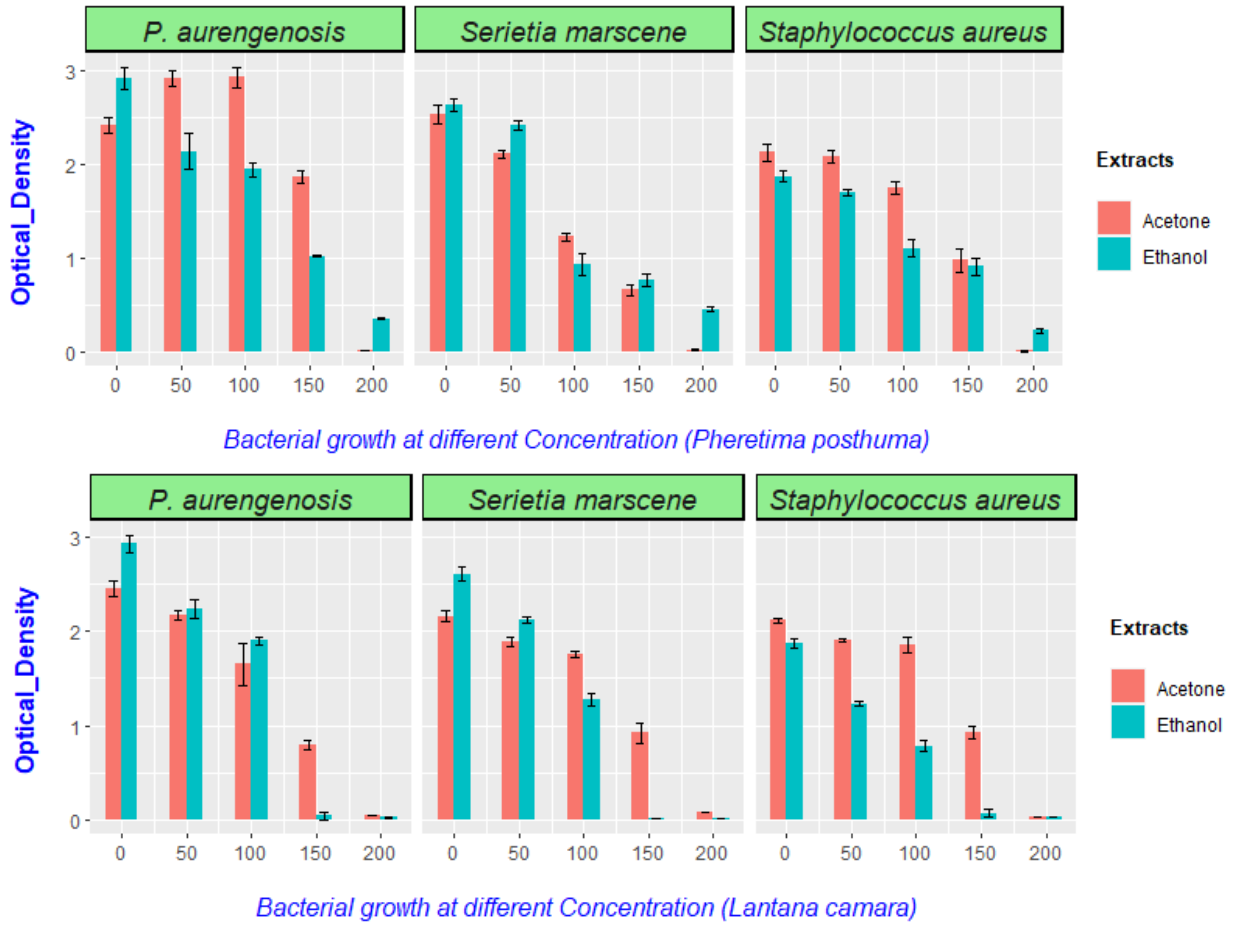


Figure 3 Graphs show the growth of strains at different concentrations of each extract

Ethanol extract of *L. camara* showed the largest growth inhibitory zone against *P. aeruginosa*, *Serratia marcescens*, then *Staphylococcus aureus.* It indicates *L. camara* contains large organic compounds soluble in ethanol, potent candidates for antibacterial growth against these strains. Acetone extract showed a growth inhibitory zone against all bacterial strains. However, their zone of inhibition was smaller than ethanol, indicating a decrease in inhibitory capacity against selected strains of bacteria compared to ethanol extract. Ethanol extract of earthworm showed inhibition against *Serratia marcescens* and *Pseudomonas aeruginosa* followed by *Staphylococcus aureus*.

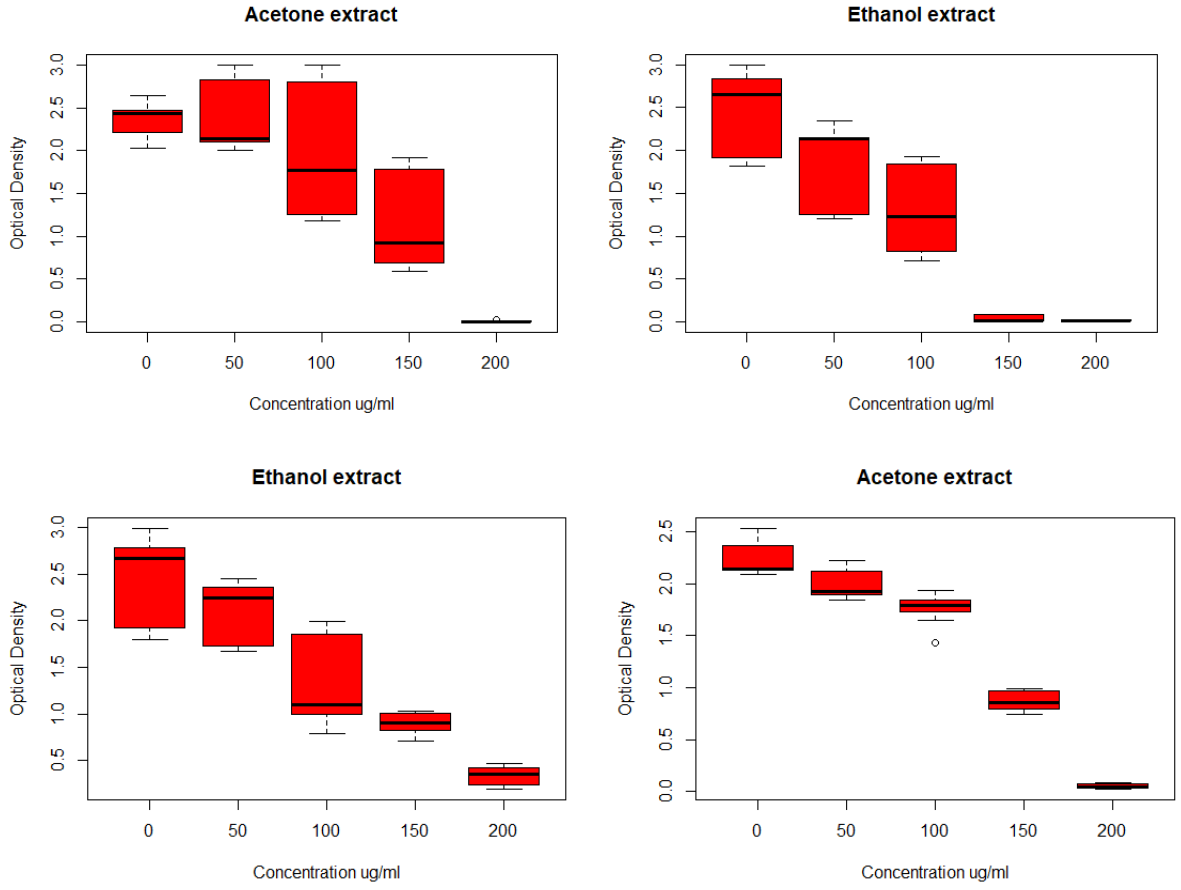


Figure 4 plotted mean growth values against antibacterial extracts

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 Dunnett's test for comparing several treatments with a control
    95% family-wise confidence level

$`0`
              diff     lwr.ci     upr.ci    pval
50-0   0.008777778 -0.5511306  0.5686861  1.0000
100-0 -0.391900000 -0.9518083  0.1680083  0.2401
150-0 -1.195888889 -1.7557972 -0.6359806 8.6e-06 ***
200-0 -2.353190000 -2.9130983 -1.7932817 4.0e-15 ***

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Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
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Figure 5 showing results of confidence level for acetone extract of earthworm

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Analysis of variance (One way ANOVA) and post-hock test was used to evaluate the difference of mean between control and treated groups for statistical significance of the data ($p \leq 0.05$). Our results at different concentrations demonstrated that MIC for both organic strains was 150µg/ml, but excellent efficiency at 200µg/ml with zero growth seems to be

bactericidal activity. *L. camara* ethanol extract showed the highest antibacterial activity compared to acetone extract and ethanol extract of earthworms. Results shown for *S. aureus were* the most important because of the increasing difficulty of treating *S. aureus* infection due to antibiotic resistance (Cruz et al., 2005). The second most important result is the inhibition of *P. aeruginosa*, which causes wound infections, especially severe burns (Yau, Ho, Tan, Ng, & Ding, 2001). This study supports the antibacterial activity of *L. camara* for developing ointment to treat wound infections.

Conclusion

Ethanol extract from the plant (*Lantana Camara*) leaves exhibited the highest antibacterial activity than acetone extract, followed by good antibacterial activity of ethanol extract of earthworm skin (*Pheretima posthuman)*, then acetone extract of earthworm (*Pheretima posthuman).* This study supports the use of *Lantana camara* extract for the development of ointment for the treatment of wound infection.

Conflict of interest

The authors declared absence of conflict of interest.

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