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*Full Length Research Paper*

# The phytochemical, antibacterial and antioxidant activity of five medicinal plants against the wound infecting bacteria

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**Leaf extracts of *Senna italica, Ricinus communis, Lantana camara, Lippia javanica* and *Ziziphus mucronata* were screened for biological activity against bacteria which infect wounds. The leaves were extracted using different solvents of varying polarity (hexane, dichloromethane, acetone and methanol). Phytochemical analyses of the extracts were performed using thin layer chromatography (TLC). The extracts were loaded on TLC plates and developed in three solvent systems that is benzene/ethanol/ammonium solution (BEA), chloroform/ethyl acetate/formic acid (CEF) and ethyl acetate/methanol/water (EMW). Antibacterial activity of the plants was evaluated using micro-dilution and bioautography methods. The test organisms used were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis.* Acetone extracts were chosen for antioxidant activity. Methanol was the best extractant, followed by acetone and dichloromethane (DCM). In the phytochemical analysis, more compounds were observed on BEA, followed by EMW and CEF plates. *Lantana camara* had no activity against any of the bacteria used. *P. aeruginosa* was the most resistant bacterium with only two plants active against it. *E. faecalis* and *E. coli* were sensitive to the extracts. More antibacterial compounds were observed on BEA plates against all the test bacteria in bioautographic method. The $R_f$ values calculated from bioautography indicated that the selected plants have different active compounds. The most active compounds were from *S. italica and Z. mucronata.* BEA and EMW plates had good antioxidant activity. No antioxidant activity was observed on the CEF plate. Most extracts were active against wound pathogens; their application on the wound area may prevent infection. Further studies are required to identify the active compounds in the plant extracts which showed significant anti-bacterial activities.**

**Key words:** Thin layer chromatography (TLC), plant extract, bacteria.

## INTRODUCTION

Since the beginning of time, man has been dependant on plants for medicinal purposes. Plants have been used to treat a variety of ailments and the introduction of orthodox medicine did not affect their use (Chah et al., 2006). Currently, it is estimated that 80% of the world's population is still dependant on plants for their health needs. About 60% of the people in South Africa use plants in conjunction with pharmaceuticals (McGaw and Eloff, 2008). In many under developed and developing countries, rural people are still forced to use traditional medicine due to inaccessibility of healthcare facilities, escalating costs of modern medicine and prior knowledge

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of plants used to treat particular ailments (Muthu et al., 2006; Steenkamp et al., 2004). Furthermore, pathogens have acquired resistance towards many antibiotics. Some antibiotics, while effective, have been associated with adverse effects such as hypersensitivity, immune-suppression and allergic reactions (Ahmad et al., 1998). There is an increased demand for cheap medicines in South Africa due to high unemployment rate and in-creased human immune virus (HIV) infections, resulting in increase in the sale of medicinal plant material (Shai et al., 2008).

Many plants produce secondary metabolites which act against wound-infecting bacteria and parasites (McGaw and Eloff, 2008). Plants are a good source of antioxidants and also cure various disorders associated with wound inflammation (Mathur et al., 2011). Identification and studies on active compounds from plants are now of major interest to the scientific community (Shai et al., 2008). These include compounds such as diterpenoid found in *Jatropha zeyheri* which showed antibacterial activity against *Streptococcus pyogenes* and some fungi. Tannins, found in a number of plants used in wound healing were also found to have antibacterial activity (Luseba et al., 2007).

There are several of bacteria which are responsible for wound infections resulting in derailed wound healing. Some bacterial wound infections can be characterized by a change in the colour of the skin around the wound area and formation of lesions. One such infection is that of *Pseudomonas aeruginosa*, characterized by a green pigment which later becomes a black lesion (Al-Akayleh, 1999). Pathogenic bacterium such as *Staphylococcus aureus* has been isolated from diabetic wounds and foot ulcers (Hirsh et al., 2008). *Enterococcus faecalis* have also been isolated from surgical wounds (Giacometti et al., 2000). Todar (2007) found extraintestinal *Escherichia coli* in surgical wounds. This study aimed at evaluating the phytochemical, antibacterial and antioxidant activity of *Lantana camara, Lippia javanica, Ricinus communis, Senna italica* and *Ziziphus mucronata* against bacteria which infect wounds (Table 1).

## MATERIALS AND METHODS

### Plant collection

The leaves of *L. camara, L. javanica, R. communis, S. italica* and *Z. mucronata,* respectively were collected at the University of Limpopo and were stored at room temperature in a well ventilated room. The leaves were ground to powder using mortar and pestle. The ground leaves were stored in air-tight containers until needed.

### Organisms used

*S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922 species are the major cause of nosocomial infections in hospitals (Sacho and Schoub, 1993) and are mainly the strains recommended for use by the National Committee for Clinical Laboratory Standards (NCCLS, 1992).

### Extraction procedure

The ground leaves (1 g) were separately extracted with 10 ml of hexane, dichloromethane, acetone and methanol, using 50 ml centrifuge tubes. Extraction was performed three times per leaf sample. The extracts were filtered through Whatman no. 1 filter paper into universal bottles. The solvents were evaporated by placing the filtrates under a fan.

### Phytochemical analysis

The plant extracts were dissolved in acetone to give a final concentration of 10 mg/ml. For each plant 10 µl (100 µg) was loaded on aluminium-backed thin layer chromatography (TLC) plate (Sigma) and the plate was developed in three solvent systems that is benzene/ethanol/ammonium solution (18:2:0.2) [BEA] (non polar/basic): chloroform/ethyl acetate/formic acid (10:8:2) [CEF] (intermediate polarity/acidic): ethyl acetate/methanol/water (10:1.35:1) [EMW] (polar/neutral) (Kotze and Eloff, 2002). The plates were viewed under ultra violet (UV) light (254 and 365 nm) for compounds which are fluorescent and later sprayed with vanillin (Sigma) sulphuric acid reagent and heated to visualize colors of the different compounds in each extract (Figure 2).

### Antibacterial activity

#### *Quantitative antibacterial activity assay by minimum inhibitory concentration (MIC)*

The serial microplate dilution method developed by Eloff (1998a) was used to determine minimum inhibitory concentration (MIC) values. Four bacterial species were used for the assay, *E. coli E. faecalis, P. aeruginosa and S. aureus.* Each organism was inoculated into 150 ml of nutrient broth, incubated for 24 h at 37°C and was used as the stock culture. The organisms (10 ml) were further inoculated in 100 ml of nutrient broth and incubated at 37°C for 24 h. The plant residues were re-dissolved in acetone to give a final concentration of 10 mg/ml and 100 µl of each plant extract was serially diluted (50%) with water in a 96 well microtitre plate. Hundred microlitres of bacterial culture was added to each well (Table 2). The plates were incubated at 37°C for 24 h. Similar dilution of ampicillin (Sigma) was used as the positive control. After incubation, 40 µl of ρ-iodinitrotetrazolium violet (INT) (Sigma) dissolved in water was added to each of the microplate wells to detect growth. Bacterial growth inhibition was indicated by the reduction of the purple color. The total activity of the extracts in ml/g was calculated by dividing the MIC value with the quantity extracted from 1 g of plant material (Table 3). The resultant value indicates the volume to which the extract can be diluted and still inhibit the growth of a microorganisms (Eloff, 2004).

#### *Qualitative antibacterial activity assay by bioautography*

The bioautography procedure described by Begue and Kline (1972) was used to determine the number of antibacterial compounds. The TLC plates were loaded with 20 µl (200 µg) of each plant extract dissolved in acetone. The plates were developed in solvent systems as used in phytochemical analysis. After development the plates were placed under a stream of air for a period of 5 days to allow the solvents to evaporate. The plates were sprayed with the various cultures, separately, until the plates were just wet and then incubated at 37°C for 24 h. The plates were then sprayed with INT

**Table 1.** Plants used in this study and their applications.

| Botanical name | Family | Application |
|---|---|---|
| *Ricinus communis* | Euphorbiaceae | Used to treat warts, cold tumours, indurations of the abdominal organs etc. (Carvalha et al., 1997). |
| *Ziziphus mucronata* | Rhamnaceae | Root and leave pastes applied on boils, swollen glands, wounds and sores (Mthethwa et al., 2009). |
| *Lippia javanica* | Verbenaceae | Used to treat skin rashes, stings and bites (Bussmann et al., 2006). |
| *Lantana camara* | Verbenaceae | Used to treat itches, cuts, ulcers, swelling, and eczema and wound healing (Raina et al., 2008). |
| *Senna italic* | Fabaceae | Leaves used as dressing for skin problems such as burns and ulcers (Masoko et al., 2010). |

**Table 2.** MIC values of the selected plant extracts in mg/ml.

| Organism | *S. italica* | *L. javanica* | *R. communis* | *Z. mucronata* | *L. camara* | *Ampicillin* (µg/ml) |
|---|---|---|---|---|---|---|
| *E.coli* | 1.25 | 0.64 | 1.25 | 0.53 | - | 0.16 |
| *E. faecalis* | 1.25 | 0.64 | 0.84 | 1.05 | - | 0.16 |
| *P.aeruginosa* | 0.84 | 0.32 | - | - | - | 0.13 |
| *S. aureus* | 0.63 | 0.64 | - | 0.53 | - | 0.08 |
| Average | 0.99 | 0.56 | 1.05 | 0.70 | - | 0.13 |

- indicates no activity.

**Table 3.** Total activity of the selected plant extracts (ml). Total activity was calculated as the mass of the plant extract divided by the MIC value.

| Organism | *S. italica* | *L. javanica* | *R. communis* | *Z. mucronata* | *L. camara* |
|---|---|---|---|---|---|
| *E.coli* | 20 | 127 | 77 | 60 | - |
| *E. faecalis* | 20 | 127 | 114 | 30 | - |
| *P.aeruginosa* | 30 | 253 | - | - | - |
| *S. aureus* | 40 | 127 | - | 60 | - |
| Average | 28 | 159 | 96 | 50 | - |

- indicates no activity.

(concentration) and incubated for 30 min. The presence of clear bands on the plates against a purple background indicated growth inhibition (Begue and Kline, 1972).

**Qualitative 2, 2-diphenyl-1-pacrylhydrazyl (DPPH) assay on TLC**

TLC plates were used to separate extracts as described. The plates were dried in the fume-hood. To detect antioxidant activity, chromatograms were sprayed with 0.2% 2,2-diphenyl-2-picrylhydrazyl (DPPH) (Sigma) in methanol, as an indicator. The presence of antioxidant compounds was detected by yellow spots against a purple background on TLC plates sprayed with 0.2% DPPH in methanol.

## RESULTS AND DISCUSSION

Majority of traditional healers use readily available water for extraction since it is readily available and it is non-toxic. In this study, the extraction process was carried out using different solvents. The problem with using water is that non-polar active compounds cannot be extracted (Masoko et al., 2008). All the extracts were placed under a fan to evaporate the solvents, after which the extracts were re-dissolved in acetone because acetone has been found to be a good solvent for both polar and non-polar compounds and it has been demonstrated that under similar test conditions not harmful to organisms such as

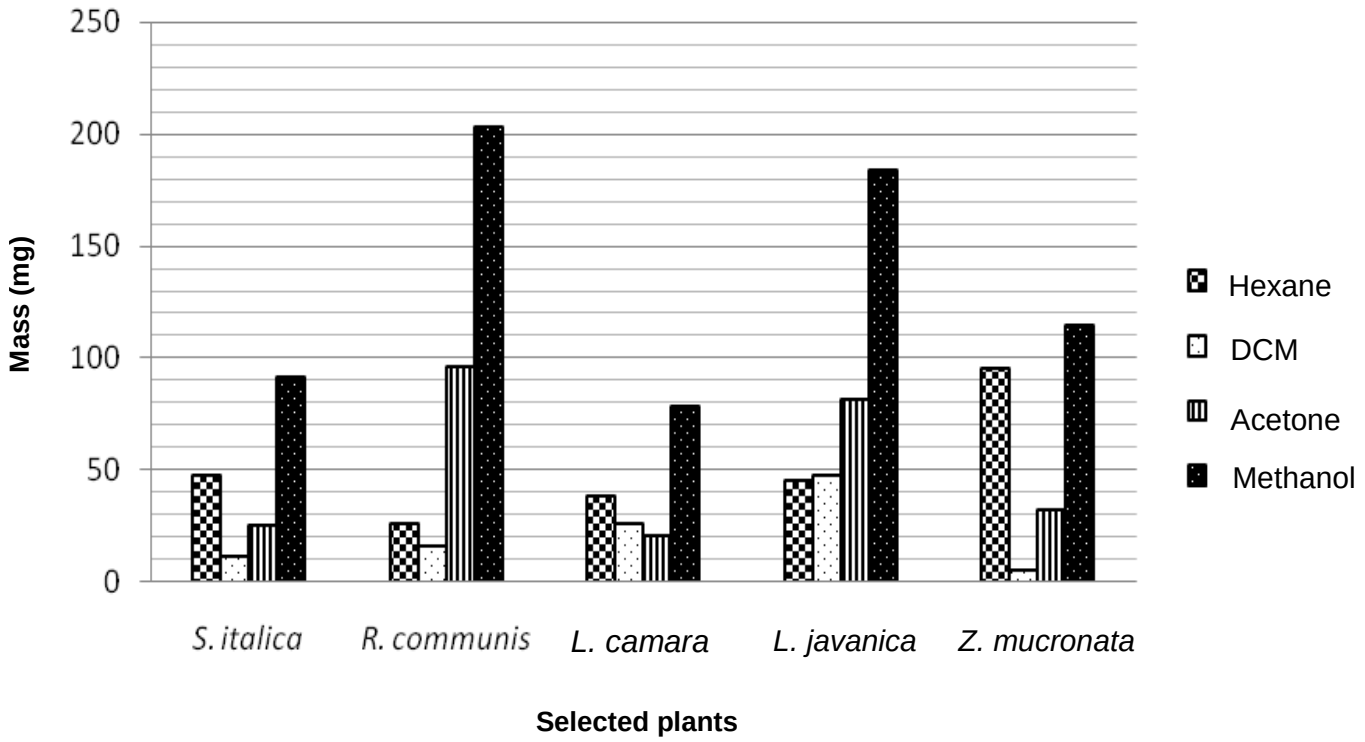


**Figure 1.** The mass of the plants extracted using different solvents (extraction process).

fungi (Masoko et al., 2007) and bacteria (Eloff, 1998b). Methanol was found to be the best extractant for all the plants since it extracted the greatest mass (Figure 1). In the phytochemical analysis, more bands were observed in BEA, followed by CEF and EMW (Figure 2) which shows that most compounds are non-polar.

*L. javanica* extract with an overall average MIC value of 0.56 mg/ml was the most active. *R. communis* had the highest MIC average of 1.05 mg/ml and was only active against two bacterial species, namely *E. coli* and *E. faecalis*. This contradicted the findings on *R. communis* by Luseba et al. (2007) which showed good MIC values against *S. aureus, E. coli* and *P. aeruginosa.* They attributed the good activity to the presence of compounds such as tannins, flavonoids and indole-3-acetic acid (Luseba et al., 2007). In this study, *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) were the most sensitive, and *P. aeruginosa* (Gram-negative) the most resistant. *P. aeruginosa* is also associated with nosocomial infections and is difficult to eradicate due to its resistance to most antimicrobial agents (Todar, 2007). *L. camara* was not active against any of the organisms studied. Previous studies have shown that Gram-positive bacteria are most susceptible to extracts (Vlietinck et al., 1995).

From the bioautograms (Figure 3), *S. italica* and *Z. mucronata* had a high number of active compounds in BEA. CEF either had a few bands or there was no bacterial growth due to formic acid. Masoko et al. (2008) reported that formic acid inhibit the growth of certain microorganisms. Preliminary screening studies by McGaw et al. (2007) using *Z. mucronata* extracts showed high levels of antibacterial efficacy. The compound 2,3-dihydroxyl-up-20-en-28-oic acid was isolated from the plant and showed excellent activity against *S. aureus* which supports claims about the efficacy of *Z. mucronata* leaf paste in treating bacterial infections in both humans and animals (McGaw and Eloff, 2008). A literature survey on the chemical constituents of the genus *Senna* revealed the presence of alkaloids, quinines and anthraquinones (Masoko et al., 2010), and these compounds are suspected to be responsible for the biological activity of the plants in this genus. Previous studies also showed that 2,5-dihydroxy-3-methoxy-7-methylanthraquinone isolated from *S. italica* showed activity against several Gram positive and Gram negative bacteria (Masoko et al., 2010).

The $R_f$ values (Tables 4 to 7) of the zones of inhibition on the bioautograms were calculated. The clear zones were located at different $R_f$ values on bioautograms suggesting that more than one compound is involved in the antibacterial activity. The values in Tables 8, 9 and 10 show that some of the zones of inhibition appeared in more than one plant and even in more than one solvent system. The presence of many bands in BEA shown in Table 4, suggests that nonpolar compounds have more

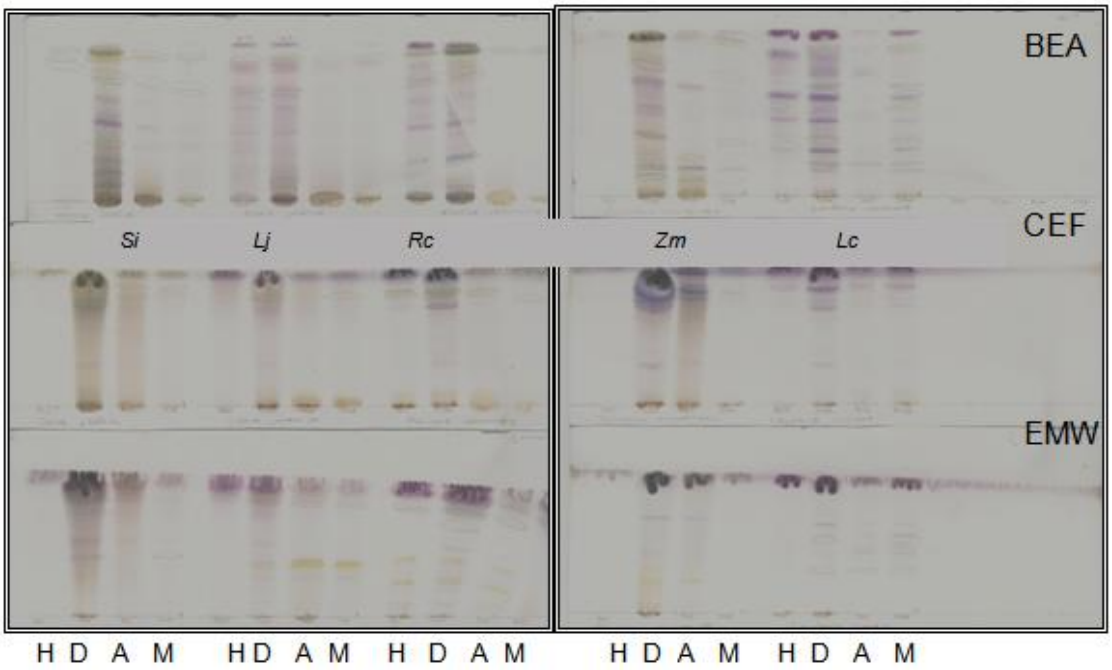


**Figure 2.** Chromatograms sprayed with vanillin sulphuric-acid reagent to show compounds extracted with hexane (H), dichloromethane (D), acetone (A) and methanol (M) from *Senna italica (Si); Lippia javanica (Lj); Ricinus communis (Rc); Ziziphus mucronata (Zm)* and *Lantana camara (Lc)* (phytochemical analysis).

**Table 4.** The inhibition of bacterial growth by bioautography of the extracts of *S.italica*, *L. javanica* and *R. communis* separated by TLC with BEA as eluent. $R_f$ values and relative degree of inhibition.

| $R_f$ value | BEA/ Hexane | | | | BEA/DCM | | | | BEA/ Acetone | | | | BEA/ Methanol | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a |
| | | | | | | | ***Senna italica*** | | | | | | | | | |
| 0.77 | | | | | | | | X | | | | | | | | |
| 0.72 | | | | | | | XXX | XXX | | | | X | | | | |
| 0.56 | | | | | XXX | XXX | XXX | XXX | | | | | | | | |
| 0.46 | XX | | | | XXX | | | | X | | | | XX | XXX | | |
| 0.31 | | | | | XXX | XXX | | | | | | | | | | |
| 0.21 | | | | | XXX | | XXX | XXX | | | | | | | | |
| | | | | | | | ***Lippia javanica*** | | | | | | | | | |
| 0.56 | | | | | | | XXX | | | | | | | | | |
| 0.46 | XX | | | | XX | | | XX | | | | | | | | |
| 0.31 | | X | XX | | XX | X | XX | X | | | | | | | | |
| 0.21 | | XXX | | | | X | | | | | | | | | | |
| | | | | | | | ***Ricinus communis*** | | | | | | | | | |
| 0.77 | | | | X | | | XXX | X | | | | | | | | |
| 0.69 | | | | | | | XX | X | | | | | | | | |
| 0.51 | | XX | | XX | | XX | XX | XXX | | | | | | | | |
| 0.46 | | | | | XX | | | | | | | | | | | |
| 0.38 | | | | | XXX | | | | | | | | | | | |
| 0.31 | XXX | X | | | XXX | XXX | | X | | | | | | | | |
| 0.21 | | XXX | X | | | XXX | X | X | | X | | | | | | |

E.c = *E. coli*, E.f = *E. faecalis*, S.a = *S. aureus*, P.a = *P. aeruginosa*. Relative degree of inhibition: X = slight inhibition, XX = moderate inhibition, XXX = high inhibition. BEA = benzene:ethanol:ammonium solution.

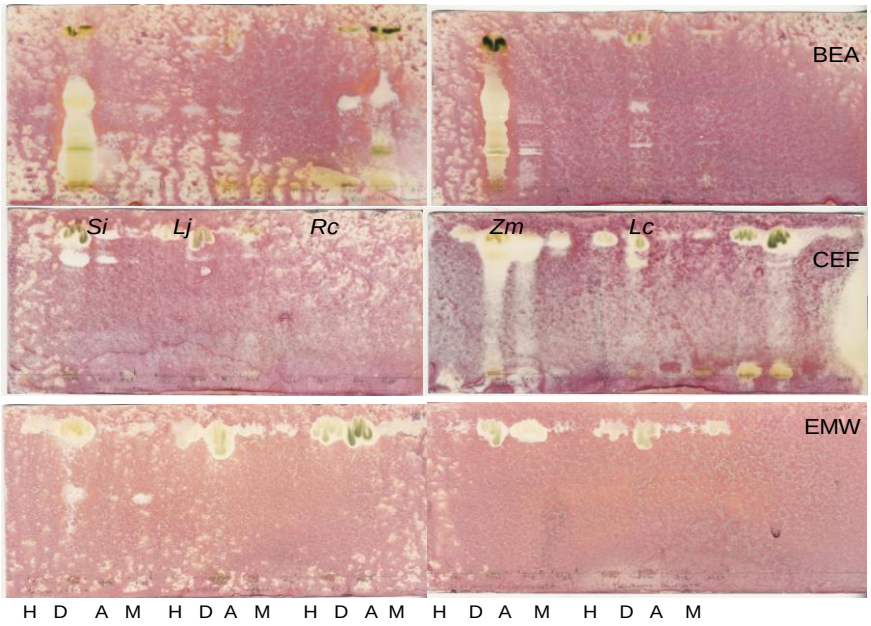


**Figure 3.** Bioautograms of selected plant extracted with hexane (H), dichloromethane (D), acetone (A), methanol (M), separated by BEA, CEF, and EMW and sprayed with *E. coli.* White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds which inhibited the growth of *E. coli* (bioautography).

**Table 5.** The inhibition of bacterial growth by bioautography of the extracts of *Z. mucronata* and *L. camara* separated by TLC with BEA as eluent. $R_f$ values and relative degree of inhibition.

| | BEA/ Hexane | | | | BEA/DCM | | | | BEA/ Acetone | | | | BEA/ Methanol | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **$R_f$values** | **E.c** | **E.f** | **S.a** | **P.a** | **E.c** | **E.f** | **S.a** | **P.a** | **E.c** | **E.f** | **S.a** | **P.a** | **E.c** | **E.f** | **S.a** | **P.a** |
| ***Ziziphus mucronata*** | | | | | | | | | | | | | | | | |
| 0.81 | | | | | | | | XX | | | | | | | | |
| 0.7 | | | | | | | | XX | | | | | | | | |
| 0.57 | | | | | | | | XX | | | | | | | | |
| 0.48 | | | | | XXX | | XXX | | X | | XXX | | | | | |
| 0.42 | | | | | XXX | | | | XX | | | | | | | |
| 0.38 | | | | | | XXX | XXX | | | XXX | | | | X | | |
| 0.35 | | | | | XXX | XXX | | | | XXX | | | | | | |
| 0.26 | | | | | XXX | XXX | XXX | XXX | | XXX | | XXX | | | | |
| 0.19 | | | | | XXX | | | XXX | | | XX | XXX | | | | X |
| 0.16 | | | | | XXX | | XXX | XXX | | | | | | | | |
| 0.13 | | | | | XXX | XXX | | XXX | | | XX | XXX | | X | | XX |
| ***Lantana camara*** | | | | | | | | | | | | | | | | |
| 0.97 | | | X | | | | X | | | | | | | | | |
| 0.86 | | | | | | | X | | | | | | | | | |
| 0.63 | | X | | | | XXX | X | X | | | | | | X | | |
| 0.51 | | | | X | | XX | X | XX | | | | | | X | | X |
| 0.48 | | | | X | XXX | | | XX | | | | | X | | | XX |
| 0.38 | | | | X | X | | | XX | | | | X | X | | | X |
| 0.26 | | | | | X | | | | | | | | | X | | |
| 0.13 | | X | X | | | XXX | X | | | | | | | | | |

E.c = *E. coli*, E.f = *E. faecalis*, S.a = *S. aureus*, P.a = *P. aeruginosa*. Relative degree of inhibition: X = slight inhibition, XX = moderate inhibition, XXX = high inhibition. BEA = benzene:ethanol:ammonium solution.

**Table 6.** The inhibition of bacterial growth by bioautography of the extracts of the selected plants separated by TLC with CEF as eluent. $R_f$ values and relative degree of inhibition.

| $R_f$ value | CEF/Hexane | | | | CEF/DCM | | | | CEF/Acetone | | | | CEF/Methanol | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a |
| ***Senna italica*** | | | | | | | | | | | | | | | | |
| 0.73 | | | | | XXX | | | | XXX | | | | X | | | |
| 0.62 | | | | | | XXX | | | | XXX | | | | | | |
| 0.21 | | | | | | XXX | | | | XXX | | | | XXX | | |
| ***Lippia javanica*** | | | | | | | | | | | | | | | | |
| 0.73 | | | | | XX | | | | X | | | | XX | | | |
| 0.69 | | | | | | | | XXX | | | | | | | | |
| 0.62 | | | | | | XX | | | | | | | | | | |
| ***Ricinus communis*** | | | | | | | | | | | | | | | | |
| 0.72 | | | | | | XXX | | | | | | | | | | |
| 0.62 | | | | | | XXX | | | | | | | | | | |
| ***Ziziphus mucronata*** | | | | | | | | | | | | | | | | |
| 0.82 | | | | | | XXX | | | | XXX | | | | XX | | |
| 0.72 | | | | | | | XXX | | | | XXX | | | | | |
| 0.67 | | | | | | | XXX | | | | XXX | | | | | |
| 0.62 | | | | | | | XXX | | | | XXX | | | | X | |
| 0.56 | | | | | XXX | | | | | | | | | | | |
| ***Lantana camara*** | | | | | | | | | | | | | | | | |
| 0.72 | | | | | | | X | | | | | | | | | |
| 0.56 | | | | | XX | | | | | | | | | | | |

E.c = *E. coli*, E.f = *E. faecalis*, S.a = *S. aureus*, P.a = *P. aeruginosa*. Relative degree of inhibition: X = slight inhibition, XX = moderate inhibition, XXX = high inhibition. CEF = chloroform:ethylacetate:formic acid.

**Table 7.** The inhibition of bacterial growth by bioautography of the extracts of the selected plants separated by TLC with EMW as eluent. $R_f$ values and relative degree of inhibition.

| $R_f$ values | EMW/Hexane | | | | EMW/DCM | | | | EMW/Acetone | | | | EMW/Methanol | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a |
| ***Senna italica*** | | | | | | | | | | | | | | | | |
| 0.71 | | | | | XXX | | | | X | | | | XXX | | | |
| 0.63 | | | | XX | | XXX | | XXX | | X | | X | | XXX | | XXX |
| 0.4 | | | | | | | XXX | | | | | | | | | |
| ***Lippia javanica*** | | | | | | | | | | | | | | | | |
| 0.56 | | | | | | XX | XX | XX | | | | | | | | |
| ***Ricinus communis*** | | | | | | | | | | | | | | | | |
| 0.53 | | | | | | XX | XX | XX | | | | | | | | |
| 0.46 | | XX | XX | XX | | XXX | XXX | XXX | | | | | | | | |
| ***Lantana camara*** | | | | | | | | | | | | | | | | |
| 0.44 | | | | | | X | XX | | | X | | | | X | XX | |
| 0.31 | | | | | | | XX | | | | | | | XX | XX | |
| 0.18 | | | | | | | | | | | | | | | XX | |

E.c = *E. coli*, E.f = *E. faecalis*, S.a = *S. aureus*, P.a = *P. aeruginosa*. Relative degree of inhibition: X = slight inhibition, XX = moderate inhibition, XXX = high inhibition. EMW = ethyl acetate:methanol:water.

**Table 8.** Number of antibacterial bands at different $R_f$ values in all the five selected plants separated by BEA.

| $R_f$ value | BEA/Hexane | | | | BEA/DCM | | | | BEA/Acetone | | | | BEA/Methanol | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | |
| 0.97 | - | - | - | 1 | - | - | 1 | - | - | - | - | - | - | - | - | - | 2 |
| 0.86 | - | - | - | - | - | - | 1 | - | - | - | - | 1 | - | - | - | - | 2 |
| 0.81 | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | 1 |
| 0.77 | - | - | - | 1 | - | - | 1 | 2 | - | - | - | - | - | - | - | - | 4 |
| 0.72 | - | - | - | - | - | - | 1 | 1 | - | - | - | 1 | - | - | - | - | 3 |
| 0.7 | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | 1 |
| 0.69 | - | - | - | - | - | - | 1 | 1 | - | - | - | - | - | - | - | - | 2 |
| 0.63 | - | 1 | - | - | - | 1 | 1 | 1 | - | - | - | - | - | - | 1 | - | 5 |
| 0.57 | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | 1 |
| 0.56 | - | - | - | - | 1 | 1 | 2 | 1 | - | - | - | - | - | - | - | - | 5 |
| 0.51 | - | 1 | - | 2 | - | 2 | 2 | 2 | - | - | - | - | - | - | 1 | 1 | 11 |
| 0.48 | - | - | - | - | 2 | - | 1 | 1 | 1 | - | 1 | - | - | - | - | 1 | 7 |
| 0.46 | 2 | - | - | - | 3 | - | - | 1 | 1 | - | - | - | 1 | 1 | - | - | 9 |
| 0.38 | - | - | - | 1 | 2 | - | - | 1 | - | - | 1 | - | 1 | 1 | - | 1 | 8 |
| 0.35 | - | - | - | - | 1 | 1 | - | - | - | 1 | - | 1 | - | - | - | - | 4 |
| 0.31 | 1 | 2 | 1 | - | 3 | 3 | - | 2 | 1 | - | - | - | - | - | - | - | 13 |
| 0.26 | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | 1 |
| 0.21 | - | 2 | 1 | - | 1 | 2 | 2 | 2 | - | 1 | - | - | - | - | - | - | 11 |
| 0.19 | - | - | - | - | 1 | - | - | 1 | - | - | 1 | 1 | - | - | - | - | 4 |
| 0.16 | - | - | - | - | 1 | - | 1 | 1 | - | - | - | - | - | - | - | - | 3 |
| 0.13 | - | 1 | 1 | - | - | - | - | - | - | - | 1 | 1 | - | - | - | - | 4 |
| Total | 3 | 7 | 3 | 5 | 16 | 10 | 14 | 20 | 3 | 2 | 4 | 5 | 2 | 2 | 2 | 3 | |
| Grand total | 18 | | | | 60 | | | | 14 | | | | 9 | | | | |

E.c = *E. coli*, E.f = *E. faecalis*, S.a = *S. aureus*, P.a = *P. aeruginosa*. Relative degree of inhibition: X = slight inhibition, XX = moderate inhibition, XXX = high inhibition. BEA = Benzene:ethanol:ammonium.

**Table 9.** Number of antibacterial bands at different $R_f$ values in all five selected plants separated by CEF.

| $R_f$ value | CEF/Hexane | | | | CEF/DCM | | | | CEF/Acetone | | | | CEF/Methanol | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | |
| 0.82 | - | - | - | - | - | 1 | - | - | - | 1 | - | - | - | 1 | - | - | 3 |
| 0.73 | - | - | - | - | 2 | - | - | - | 2 | - | - | - | 2 | - | - | - | 6 |
| 0.72 | - | - | - | - | - | 1 | 1 | - | - | - | 1 | - | - | - | - | - | 3 |
| 0.69 | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | 1 |
| 0.67 | - | - | - | - | - | - | 1 | - | - | - | 1 | - | - | - | - | - | 2 |
| 0.62 | - | - | - | - | - | 3 | 1 | - | - | 1 | 1 | - | - | - | 1 | - | 7 |
| 0.56 | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | 1 |
| 0.21 | - | - | - | - | - | 1 | - | - | - | 1 | - | - | - | 1 | - | - | |
| Total | 0 | 0 | 0 | 0 | 3 | 6 | 3 | 1 | 2 | 3 | 3 | 0 | 2 | 2 | 1 | 0 | 3 |
| Grand total | 0 | | | | 13 | | | | 8 | | | | 5 | | | | |

E.c = *E. coli*, E.f = *E. faecalis*, S.a = *S. aureus*, P.a = *P. aeruginosa*. Relative degree of inhibition: X = slight inhibition, XX = moderate inhibition, XXX = high inhibition. CEF = chloroform:ethylacetate:formic acid.

antibacterial activity. More zones of inhibitions were observed in DCM, suggesting that the solvent was able to extract many active compounds. The non-activity of the polar extracts in bioautography is thought to be attributed to the evaporation of volatile active compounds during removal of the TLC eluents or disruption of synergism between active constituents caused by TLC separation (Masoko and Eloff, 2006). The DPPH method measures

**Table 10.** Number of antibacterial bands at different $R_f$ values in all five selected plants separated by EMW.

| $R_f$ value | EMW/Hexane | | | | EMW/DCM | | | | EMW/Acetone | | | | EMW/Methanol | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | E.c | E.f | S.a | P.a | |
| 0.71 | - | - | - | - | 1 | - | - | - | 1 | - | - | - | 1 | - | - | - | 3 |
| 0.63 | - | - | - | 1 | - | 1 | - | 1 | - | 1 | - | 1 | - | 1 | - | 1 | 7 |
| 0.56 | - | - | - | - | - | 1 | 1 | 1 | - | - | - | - | - | - | - | - | 3 |
| 0.53 | - | - | - | - | - | 1 | 1 | 1 | - | - | - | - | - | - | - | - | 3 |
| 0.46 | - | 1 | 1 | 1 | - | 1 | 1 | 1 | - | - | - | - | - | - | - | - | 6 |
| 0.44 | - | - | - | - | - | 1 | 1 | - | - | 1 | - | - | - | 1 | 1 | - | 5 |
| 0.4 | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | 1 |
| 0.31 | - | - | - | - | - | - | 1 | - | - | - | - | - | - | 1 | 1 | - | 3 |
| 0.18 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | - | 1 |
| Total | 0 | 1 | 1 | 2 | 1 | 5 | 6 | 4 | 1 | 2 | 0 | 1 | 1 | 3 | 3 | 1 | |
| Grand total | 4 | | | | 16 | | | | 4 | | | | 8 | | | | |

E.c = *E. coli*, E.f = *E. faecalis*, S.a = *S. aureus*, P.a = *P. aeruginosa*. Relative degree of inhibition: X = slight inhibition, XX = moderate inhibition, XXX = high inhibition. EMW = ethyl acetate:methanol:water.

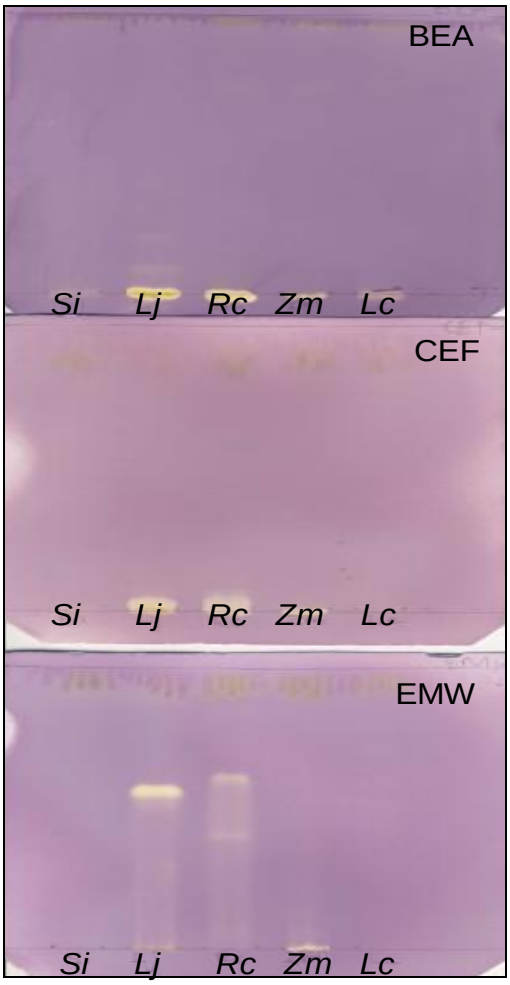


**Figure 4.** Chromatograms of the selected plants extracts separated by BEA, CEF and EMW solvent systems and sprayed with 0.2% DPPH. Si = *Sennaitalica*; Lj = *Lippia javanica*; Rc = *Ricinus communis*; Zm = *Ziziphus mucronata*; Lc = *Lantana camara* (antioxidant activity).

electron-donating activity of other compounds in the mixture and hence provides an evaluation of antioxidant activity due to free radical scavenging. Any molecule that can donate an electron or hydrogen to a mixture will react with and bleach DPPH. DPPH is reduced from a purple compound to a light yellow compound by electrons from oxidant compounds (Naik et al., 2003). The light yellow bands were observed on BEA and EMW plates. The active bands were present in *L. javanica* and *R. communis*, though not very active in BEA and in CEF no activity was observed (Figure 4) for all the plants.

## Conclusion

The results indicate that the selected plants have antioxidant activity and antibacterial activity against the test organisms. This study serves as a scientific validation for the use of the plants in traditional medicine for wound healing. Further studies to isolate the antibacterial compounds are currently underway

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