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In-Vitro Antibacterial Activity of *Lantana trifolia* Flower Extracts on Bacterial Isolates from Wounds

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ABSTRACT: This study investigated the phytochemical constituents and antibacterial activities of *Lantana trifolia* flower extracts against bacterial isolates from surgical and diabetic wounds of patients. Phytochemical investigation was done by standard procedure, antimicrobial screening by agar well diffusion method and antibiotics susceptibility of isolates by Kirby-Bauer disc diffusion test. Phytochemicals present include flavonoids, tannins, phenylethanoid glycosides, reducing sugars, terpenoids, saponins, anthraquinones alkaloids and steroids. Bacterial wound isolates were mainly gram +ve and include *Staphylococcus aureus*, *S. epidermidis, Enterococcus faecalis, Corynebacterium* sp., *Enterobacter aerogenes*, *Pseudomonas aeruginosa, Mycobacterium* sp. and *Escherichia coli.* Inhibition diameter was concentration. The range of inhibition diameter was significantly ($P<0.05$) lower in the aqueous extract (15.2±1.5 to 35.5 ±0.9 mm) than the ethanolic (28.3± 3.4 to 49.1±8.9mm). Antibacterial activity occurred at very low concentrations (18.75mg/ml) for ethanolic extract only. The most *L trifolia* extract sensitive bacterial isolate was *S. aureus* (35.5±0.9mm and 49.1±8.9mm for aqueous and ethanolic extracts respectively at 300mg/ml), while the least sensitive were *Corynebacterium* sp. (15.2±1.5mm) *and Mycobacterium* sp. (28.3±3.4) at 300mg/ml for aqueous and ethanolic extracts respectively. The minimum inhibitory concentration was lower for ethanolic extract, 18.75/ml to 37.5mg/ml than the aqueous extract (37.5 to 150mg/ml). The minimum bacterial concentration range from 37.5mg/ml to 150mg/ml for ethanolic extract and 75 to 300mg/ml for aqueous extract. *S. aureus* was the most antibiotic resistant strain (72%), while *P. aeruginosa* and *Mycobacterium* sp. were the least resistant (9.1%) strains. *L. trifolia* was more bacteriostatic than the most effective antibiotics, Ciprofloxacin, Rocephin, Nitrofurantoin, and deserves further investigation.

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Microorganisms especially bacteria are the causative agents of wound infections, which is an important cause of morbidity in surgical and diabetic patients (Giacometti *et al*., 2000; Mohammed *et al*., 2013). The widespread use of antibiotics to fight infections, has resulted in increased bacterial resistance to existing drugs, a development which threatens public health (Munita and Arias, 2016). Bacterial adaptation to the antibiotics attack, has led to immense genetic plasticity amongst the bacterial pathogens, in the form of mutational adaptations, acquisition of genetic materials or alteration of gene expression, producing resistance to virtually all antibiotics currently in use in clinical practice. The continued evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds. Two major indices that determine the potency of the prospective antimicrobial candidate are the minimum inhibitory concentration (MIC) and the minimum bacterial concentration (MBC). Minimum inhibitory concentration (MIC) is the lowest concentration of a chemical, usually a drug, which prevents visible growth of bacteria. MBC on the other hand, is the lowest concentration of an antibacterial agent required to kill a particular bacterium (Okigbo and Mmeka, 2008). The best alternative for new microbial agents with minimal side effects, are natural products of plant origin. Medicinal herbs are readily available, cheap, efficacious and quite popular in developing countries (Preethi *et al.,* 2010). *Lantana trifolia* Linn. (Family: Verbenaceae), was chosen for this work based on the ethnobotanical evidence of the plant world-wide. It is a terrestrial, evergreen aromatic and hairy shrub. Its resilient nature makes it invasive, dominating native species as a weed and widely distributed in the pantropics (Salada *et al*., 2015).It is used in many parts of the world in conventional medication for the cure of different human diseases, possessing analgesic, antimalarial, antifungal, antibacterial, enzyme inhibition, hepatotoxic, insecticidal and nematicidal properties (Sousa and Costa, 2012). It is a rich source of terpenoids and saponins (Sousa and Costa, 2012; Nalubega *et al.,* 2013). In this study, the bacterial

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spectrum of surgical and diabetic wounds were investigated, phytochemical and antibacterial screening of aqueous and ethanolic flower extracts of *L. trifolia* were conducted.

MATERIALS AND METHODS

Plant Materials: The *L. trifolia* red flowers were collected from a building site bush in the University of Nigeria, Nsukka campus and identified by the Department of Plant Science and Biotechnology in the university. The flowers were washed with clean water to remove dirt and drained, oven- dried at 45ºC for 5 days and ground into a powder using a milling machine (Noris and Poole, England). The powdered flower were kept in labelled sterile containers until when required.

Extraction of Crude plant Extracts: A modified method of Idris *et al*. (2009) was used. Four hundred gram (400g) of the ground plant material was macerated in I litre each of distilled water and 99.9/100% ethanol for 24 h. The solutions were filtered through a sieve to remove debris and the filtrates were then filtered through Whatman no. 1 filter paper. The final filtrates were evaporated in a water bath at 48°C to get the crude extracts. The crude aqueous and ethanolic extracts of the plant, were stored at 4°C until required for the phytochemical and antibacterial analysis. The 400g yielded and 119.2g and 51.2g representing 29. 8% and 12.8% yield for ethanolic and aqueous extracts respectively of *L. trifolia* after the extraction process.

Preparation of Concentration of Plant Extract: Seven hundred and fifty milligrams (750mg) of both ethanol and aqueous leaf extracts of *L. trifolia* were added to 5ml of ethanol and distilled water respectively to give a concentration of 300mg/ml. Other concentrations of 150, 75, 37.5, and 18.75mg/ml were prepared by dilution method as described by Evbuomwan *et al*. (2017).

Test Bacterial Isolates: Wound swabs were collected from 120 patients with infected diabetic wounds and surgical sites. To avoid contaminating the swab with skin flora, pus or necrotic tissue, the wound was thoroughly cleansed with 60–120 ml sterile normal saline prior to taking the sample. Sterile gauze was used to remove excess saline from the wound surface and the wound swabs were collected using sterile swab by swabbing at the middle of the wound. When there were two or more wounds in the same location, separate swabs were used for each wound. A swab moistened with sterile normal saline was rolled deep in the wounds and inserted immediately into a tube containing Stuart's transport media for preservation of microbes and then transported to the laboratory. Wound swabs were streaked on Blood Agar (BA) and MacConkey Agar (MCA) plates and incubated aerobically for 18–24 h at 37 °C. They were then observed for bacterial growth. Plates with no growth and with growth were re-incubated for another 18–48 h for isolation of bacteria that require extended incubation (slow growers) (Giacometti *et al*., 2000). Pure isolates were identified according to their morphological characteristics and reactions to biochemical tests.

Standardization of inoculum: Isolated bacteria were sub-cultured onto fresh plates of MacConkey or blood agar depending on the bacteria type, and incubated aerobically at 37°C for 18-24 h. Colonies from these plates were suspended in Mueller-Hinton broth (Oxford, UK) to a turbidity matching 0.5 McFarland standard (10^8 m cfu/ml). Mueller-Hinton agar was then used for antibacterial assay. All the broth cultures were incubated at 37°C (Aibinu *et al.,* 2007).

*Antimicrobial assay***:** After the isolation and identification of the bacteria, antibacterial screening of the aqueous and ethanolic extracts of *L. trifolia* flowers was done using agar diffusion method. 0.2mls of standard inoculum suspensions of the isolated bacteria were used. Each labelled plate was uniformly seeded with a test organism by means of sterile swab stick rolled in the Mueller-Hinton culture medium. A well of uniform size was made in each plate. 300, 150, 75, 37.5 and 18.75 mg/ml concentrations for aqueous and ethanolic extracts were dropped in each well to fullness. Each plate was kept in the refrigerator for 1 h to allow the extracts to diffuse into the culture medium, while the immediate growth of the organism was stopped from taking place. These plates were then incubated at 37°C for 48h. The zones of inhibition around the wells were measured in millimetre. Antibiotics such as Ciprofloxacin (2mls of 1% solution), distilled water and ethanol solvent with test organisms were placed in a well on each plate along with the test extracts as control. This was done in triplicates.

Determination of minimum inhibitory concentration: This was calculated in millimetres from readings on the culture plates after incubation.

*Determination of minimum bactericidal concentration***:** Doubling diffusion containing different concentrations as used in MIC determination was carried out thus: to a 0.5 ml extract, 0.5 ml of sterile distilled water was dispensed, from this test tube labelled '1', 0.5 ml of the mixture was taken and dispensed to a test tube labelled '2' containing 0.5 ml

sterile distilled water, this was done twice and from the last test tube labelled '4', 0.5 ml of the mixture was taken so that the mixture remained as 0.5 ml. This was done for the different concentrations of the plant extracts. To the tubes containing different concentrations of the extracts (300 to 18.75 mg/ml) 0.5 ml of each test organism was added. Samples were streaked from the tubes onto Mueller-Hinton agar plates to determine the minimum concentration of the extract required to kill the organisms. These concentrations were indicated by failure of the extract to kill the organisms. The lowest concentration that prevented bacterial growth after two days of incubation was recorded as minimum bactericidal concentration (Aibinu *et al.*, 2007).

Antibiotics susceptibility testing of the test organism: Antimicrobial disc tests of the isolates were performed according to the guidelines of Kirby-Bauer disk diffusion susceptibility test protocol (Hudzicki,2009) using the following antibiotic discs: Nitrofurantoin (20ug), Amoxicillin (30ug), Rocephin (25ug), Ciprofloxacin (l0ug), Augmentin (20ug), Gentamicin (10ug), Tetracycline (30ug), Chloramphenicol (25ug), Cefuroxime (l0ug), Ampicillin (30ug), and Ofloxacin (30ug), and antibiotics resistance was interpreted by diameter of inhibition zones around the antibiotic discs.

Qualitative phytochemical screening**:** The qualitative phytochemical screening was conducted using the following analytical methods; for the analysis of tannins and alkaloid, the method used was the method of Treas and Evans (1996) and Kanoman *et al.* (2014), while saponins, glycoside, carbohydrates, sugars and terpenoid were studied using the method as described by Harbourne (1984). Anthraquinone was analysed using methods described by Auwal *et al*. (2014). Steroids and acidic compounds were analysed using the methods described by Akpuaka (2009).

Data analysis: The results were presented as the mean ±SEM (standard error of mean) for each of the experiments. The test groups were compared with the negative and positive control groups using one-way analysis of variance (ANOVA) by means of Sigma Stat, Graph pad prism (Graphpad software, San Diego, CA). All data were analysed at a 95% confidence interval. P- values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening: Phytochemical screening of the flower of L. *trifolia* yielded nine (9) phytochemicals in both ethanolic and aqueous extracts. They include flavonoids, tannins, phenylethanoid glycosides reducing sugars, terpenoids, saponins, anthraquinones, alkaloids, and steroids (Table 1). Souza and Costa (2012) reported the presence of terpenoids, flavonoids, phenylethanoid glycosides, alkaloids, and tannins in *Lantana* sp., while Nalubega *et al*. (2013), reported the presence of flavone and glycosides in *L. trifolia.*

Table 1: Phytochemical analysis of *ethanol* and *aqueous extracts of L.trifolia*

Phytochemicals	Ethanol	Aqueous
Flavonoids	+++	+++
Tannins	++	+
Phenylethanoid glycosides	++	+
Reducing sugars	+	+
Terpenoids	+++	+++
Saponins	++	++
Anthraquinones	+	+
Alkaloids	+++	++
Steroids	+	+

Key:+ = Slightly present; ++ moderately present; +++ = highly present.

Tannins, alkaloids, and phenylethanoid glycosides, were highly present in the ethanolic than aqueous extract. The dissolution capacity of the different solvents affects the level of the phytochemicals extracted. Ethanol has a higher dissolution capacity than distilled water, as confirmed by the higher degree of the presence of some phytochemicals. The cytotoxic, antimicrobial and medicinal activities of these isolated phytochemicals have been well documented (Souza and Costa, 2012; Afidati, 2019). For instance, terpenoids, alkaloids and saponins are known to cause degradation of cytoplasmic membrane, both in function and structure. Damaging the cytoplasmic membrane which causes cytoplasm coagulation and increased membrane permeability leading to leakages of vital intracellular substances and reducing ATP synthesis (De Olivera *et al.*, 2013; Afidati, 2019). This process leads to cell death. Terpenoids can also cause large scale membrane thinning on lipid bilayer and exert antimicrobial properties via a membrane disruption mechanism. Consequently, terpenoids show significant pharmacological activities, such as anti-viral, anti-bacterial, anti-malarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-cancer activities (Souza and Costa, 2012). *L. trifolia* is rich in terpenoids as indicated in this study.

These properties make *L. trifolia* the ideal candidate for a novel plant-based antibacterial drug. Flavonoids inhibit membrane bound enzymes. This attribute may explain the antioxidant application of *L. trifolia,* locally practiced by indigenous cultures in Africa (Nalubega, 2013; Alonygudet *et al*., 2017), while phenylethanoid glycosides, which are antibacterial,

antitumor, antiviral, anti-inflammatory, neuro-protective, antioxidant (Ge *et al.,*2017; Kutluay *et al*., 2019).Tannins' antimicrobial activity is based on its ability to inhibit membrane bound enzymes (Anderson *et al.,* 2012).

Table 2: Bacteria isolates, number and percentage of occurrence

Bacterial Isolates	Number of Occurrence per sample	% Occurrence
Staphylococcus aureus	51	42.5
Staphylococcus epidermidis	10	8.33
Enterococcus faecalis	5	4.17
Corynebacterium sp.	3	2.5
Enterobacter aerogenes	2	1.7
Pseudomonas aeruginosa	30	25
Mycobacterium sp.	4	3.33
Escherichia coli	15	12.5

Morphological and biochemical characteristics of bacterial isolates: The screening of wounds for bacteria produced eight (8) bacteria. They include *Staphylococcus aureus*, *S. epidermidis, Enterococcus faecalis, Corynebacterium* sp., *Enterobacter aerogenes*, *Pseudomonas aeruginosa, Mycobacterium* sp. and *Escherichia coli.* Of the bacterial isolates collected per sample, *S. aureus* occurred in the highest number of patients (42.5%), while *E. aerogenes* occurred in the lowest number (1.7%) (Table 2).

A single etiologic agent was found in 80, multiple agents were found in 40 patients. Gram positive bacteria dominated the infected wounds. The gram positive bacteria were S. aureus, *S. epidermidis, E. faecalis, Corynebacterium* sp., while *E.aerogenes, P. aeruginosa, E. coli* were gram-ve bacteria. *Mycobacterium* sp. was an acid fast bacterium (Table 3). This does not agree with the report by Oshilim (2017), who isolated more gram –ve bacteria than gram +ve from wounds. Majority of the bacteria were also rods (6), as against the very few (2) cocci.

Table 3: Morphological and biochemical characteristics of the bacteria isolates.

Test and Assessment	*S. aureus*	*S. epidermidis*	*E. faecalis*	*Corynebacterium* sp.	*E. aerogenes*	*P. aeruginosa*	*Mycobacterium* sp.	*E. coli*
Gram stain	GPC	GPC	GPB	GPB	GNB	GNB	Acid fast bacilli	GNB
Morphological characteristics	Cocci	Cocci	Rods	Rods	Rods	Rods	Rods	Rods
Citrates	-	-	+	-	+	+	+	-
Oxidase	-	-	-	-	-	+	-	-
Catalase	+	+	+	+	+	+	+	-
Indole	+	+	–	-	-	-	-	+
Glucose	+	+	+	+	+	-	+	+
Lactose	+	+	+	-	+	-	+	+
H_2S	-	-	-	-	-	-	-	+
Gas	-	-	-	+	-	-	-	+

Key: + = positive; - = negative

Table 4: Antibacterial activity of flower extracts of *L. trifolia* (zones of inhibition are measured in millimetres, mm)

Drug/ Extracts	Concentrations (mg/ml)	*S. aureus*	*S. epidermidis*	*E. faecalis*	*Corynebacterium* sp.	*E. aerogenes*	*P. aeruginosa*	*Mycobacterium* sp.	*E. coli*
Ethanolic	Ethanol(-ve control)	0.0±0.0^a	0.0±0.0^a	0.2±0.0^a	0.5±0.1^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a
	300	49.1±8.9^b	38.1± 5.1^c	39.2± 5.8^c	30.3± 1.5^c	36.8±1.8^c	30.2±5.2^c	28.3±3.4^c	34.5±3.1^c
	150	28.5±5.6^c	21.3.1±3.2^d	23.4±5.2^d	21.2±2.2^d	23.4±2.6^d	27.2±1.7^d	15.1±2.5^e	24.2± 2.6^d
	75	19.2±0.0^e	13.1±2.6^e	18.1±6.1^e	15.1±2.6^e	18.4±2.6^e	18.3±3.2^e	8.1±0.0^f	15.3±0.6^e
	37.5	9.5±.1.4^f	5.3±.2.1^f	7.1±0.9^f	6.2±3.2^f	6.2±1.2^f	6.1±1.1^f	7.6±1.4^f	9.3±0.6^f
	18.75	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	4.2±0.6^f	0.0±0.0^a	0.0±0.0^a	6.0±0.0^f
Aqueous	Distilled water (-ve Control	0.0±0.0^f	0.0±0.0^a	0.1.0±0.0^f	0.2±0.0^a	0.2±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a
	300	35.5±0.9^c	24.6± 3.1^d	24.3±2.8^d	15.2± 1.5^e	18.1±0.8^e	20.1±2.4^d	21.8±3.2^d	28.5±3.1^d
	150	18.6±1.^{6d}	16.3.±3.2^d	18.4±5.2^d	9.2±0.2^f	11.3±2.6^e	11.1±0.5^e	13.2±0.7^e	19.2± 2.6^e
	75	7.0±0.0^e	10.1±2.6^e	4.0±0.0^f	0.0±0.0^a	0.0±0.0^a	5.1±0.1^f	5.6±2.2^f	11.3±0.6^e
	37.5	0±.0.0^a	8.3±.2.1^e	0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	2.1±0.1^a	5.3±0.6^f
	18.75	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a	0.0±0.0^a
Ciproflox acin (+ve control)	1% solution	32.6±1.0^c	25.03±2.1^d	25.2.1±3.2^d	25.5±2.6^d	18.2±2.5^e	23.3±6.1^d	18.8±3.4^e	26.3±7.1^d

Mean values with different alphabets as superscripts in a column differ significantly (P<0.05).

Antimicrobial activity of plant extracts: The antibacterial effects of the extracts is shown in Table 4.Both aqueous and ethanolic extracts of *L. trifolia* flower showed antibacterial activity. The inhibition diameter of both aqueous and ethanolic extracts were significantly (P<0.05) higher than those of the negative controls, especially at high concentrations. Antibacterial activity of *L. trifolia*, was significantly (P< 0.05) dependent on the nature of the solvent used for extraction and the concentration of the extracts. At the highest concentration (300mg/ml), inhibition zone diameter range was significantly (P<0.05) greater in the ethanolic extract (28.3±3.4 to 49.1±8.9mm) than the aqueous extract (15.2± 1.5 to 35.5±0.9 mm). The potency of *L. trifolia* was enhanced by the type of solvent used during the extraction process. Ethanol extracted more of the bioactive components of the plants, when compared with the aqueous extract. Both ethanolic and aqueous extracts exhibited nonselective antibacterial activity against the isolates. *S. aureus* was the most sensitive bacteria in both solvents with the widest inhibition diameter range for the ethanolic extract from 9.5±.1.4 mm at 37.5mg/ml to 49.1±8.9mm and the least sensitive was *Mycobacterium* sp. from 7.6±1.4 mm at 37.5mg/ml to 28.3±3.4mm at 300mg/ml. For the aqueous extracts, the same bacteria *S. aureus* exhibited a lower inhibition diameter range from 7.0±0.0 at75 mg/ml to 35.5±0.9mm at 300mg/ml, and the least sensitive was *Corynebacterium* sp. with an inhibition zone diameter of 9.2±0.2mm at 150mg/ml to 15.2± 1.5 at 300mg/ml) (Table 4).

At the highest concentration (300mg/ml), all the isolated test organisms, were significantly (P< 0.05) more sensitive to the ethanolic extract, haven registered higher inhibition zone diameters than Ciprofloxacin. However, for the aqueous extract, only *S. aureus* and *E. coli* were significantly (P< 0.05) more sensitive to the extract than the reference antibiotics, with a higher inhibition zone diameter (35.5±0.9 and 28.5±3.1mm) for *S. aereus* and *E. coli* respectively)than Ciprofloxacin (32.6. ± 1.0 and 26.3± 7.1 for *S. aureus* and *E. coli)*. All the other isolates, *S. epidermidis, E. faecalis Corynebacterium* sp., *E. aerogenes, P. aeruginosa,* and *Mycobacterium* sp.had inhibition diameters (≥ 15mm), lower than those exhibited by the isolates in the antibiotics (≥ 18mm). Though, this is a further confirmation of the higher potency of the ethanolic extract to the aqueous extract, this value indicates that the aqueous flower *L. trifolia* extract is also an efficient antibiotics. Any antimicrobial agent showing an Inhibition zone diameter of ≥ 15mm on a microorganism is an efficient antimicrobial agent (Hudzicki, 2009). The minimum inhibition concentration (*MIC*) *and minimum bacterial concentration* (*MBC)*: Isolates were highly sensitive to ethanolic extract of *L. trifolia* with MIC ranging from 18.75mg/ml (*E. coli* and *E. aerogenes*) to 37.5mg/ml (the rest of the isolates).On the other hand, Isolates were moderately sensitive to aqueous extract of *L. trifolia* with MIC ranging from 37.5 (*E. coli* and *Mycobacterium* sp) to 150mg/ml (*Corynebacterium* sp. and *E. aerogenes*). MBC for the ethanolic flower extract, ranged from 37.5 (*E. coli* and *E. aerogenes*) to 75mg/ml (rest of the isolated test organisms), for the aqueous extract, MBC was higher than that reported for the ethanolic extract and ranged from 75(*S. epidermidis*, *E. faecalis*, *Mycobacterium* and *E. coli*) to 300mg/ml (*Corynebacterium* sp.) (Figure1).

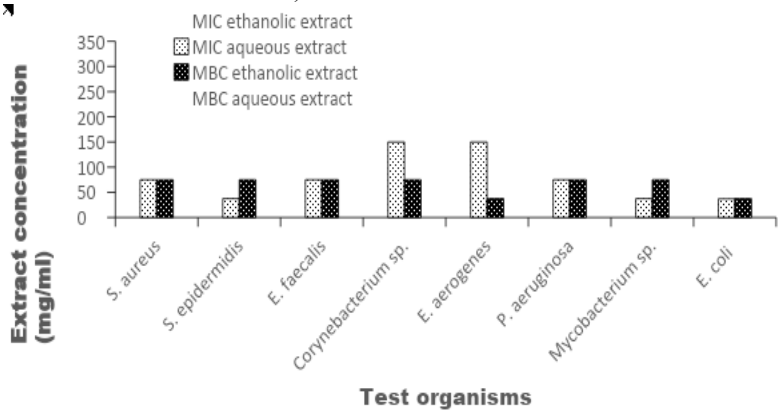

Fig 1. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of isolates to flower ethanolic and aqueous extracts of *L. trifolia*

The results from this study revealed that flower of *L. trifolia* has antimicrobial properties and therefore is a medicinal plant (Nalubega *et al.,* 2012).This report agrees with Salada *et al*. (2015) and Sharma *et al*. (2013), who reported that *Lantana* sp has antimicrobial properties. Ethanolic and aqueous

extracts of *L. trifolia* exhibited a non-selective bacteriostatic activity. The extracts exhibited antibacterial activity against both gram +ve and gram –ve bacteria making them broad spectrum. The study also revealed that *S. aureus* has a high tendency to develop resistance to antimicrobial agents. *S. aureaus* with very wide inhibition zone diameter for both aqueous and ethanolic extracts (indicates high sensitivity to the extract), however, it also had very high MIC and MBC, (indicating low sensitivity to the extract as well). These two opposing attributes indicative resistance tendency. There is need to treat *S. aureus* infections with various kinds of antibiotics, alternated after a given period of time in that environment. This is to prevent antibiotics resistance through drug pressure in *S. aureus*.

Antibiotics sensitivity: The bacterial isolates were screened for antibiotics sensitivity and exhibited varying degrees of antibiotics resistance as presented in Table 10. Eleven (11) different antibiotics were used and the organisms were selectively resistant to the antibiotics. The most resistant bacterium strain was S. aureus (72.7%), while the least resistant were *P. aeruginosa* and *Mycobacterium* sp. (9.1%). The most effective antibiotics were ciprofloxacin and Rocephin, while the least effective was tetracycline, with all isolates resistant to it (Table 5). The organisms were resistant due to plasmid borne or chromosomally mediated resistance (Munita and Arias, 2016). Comparatively, *L. trifolia* extracts were more potent antibacterial substance than the conventional antibiotics.

Table 5: Antibiotics susceptibility test

Antibiotics	*E. coli*	*Mycobacterium* sp.	*S. epidermidis*	*E. faecalis*	*Corynebacterium* sp.	*E. aerogenes*	*P. aeruginosa*	*s aureus*
Nitrofurantion	++	+++	+++	+++	+	+	+	R
Ciprofloxacin	+++	+++	++	++	++	+++	++	+
Tetracycline	R	R	R	R	R	R	R	R
Amoxicillin	R	+++	R	++	R	++	+	+
Ofloxacin	++	+++	+	R	R	R	++	R
Chloramphenicol	+	+	R	R	+	++	+	R
Cefuroxine	R	+	+	++	R	+	+	R
Ampicillin	R	+	R	+	+	R	++	R
Gentamicin	R	+++	+	++	++	++	+	R
Augumentin	R	+++	R	+	+	+	+	R
Rocephin	+	+	+	+	R	+	++	++
Resistance %	55.0	9.1	46.0	27.3	46.0	27.3	9.1	72.2

Key: +=slightly sensitive; ++ = moderately sensitive; +++ = highly sensitive; R = resistance

Conclusion: This study revealed that the flower of *L. trifolia* is a highly potent bacteriostatic plant, whose potency was enhanced using ethanol as the extraction medium. In addition, the ethanolic extract of the plant at 300mg/ml concentration, achieved antibacterial activity even higher than that of the reference antibiotics Cyprofloxacin tested. *L. trifolia* is an antimicrobial pointer to new sources of novel drugs which merits further investigation.

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