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Antimicrobial activity of Lantana camara extracts against selected clinical isolated bacteria

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***Abstract*---**Multi-drug resistance patterns in bacteria are difficult to treat. The search for a new alternative antibiotic drug that may help to control drug-resistant pathogenic bacteria is necessary. The present study focused on the antibacterial effect of Lantana camara extracts against pathogenic bacteria. Extraction was performed on L. camara leaves and stems using the maceration technique. Three extraction solvents were used. The experiments were carried in duplicate, and the results were statistically analyzed with SPSS. Tannins, phenols, saponins, alkaloids, flavonoids, and glycosides were found in L. camara plant. Methanol leaf extract possessed greater antimicrobial potential than petroleum ether and water extracts with maximum inhibition zone belonging to S. aureus 43300 (DIZ = 28 mm). The methanol stem extract of L. camara showed the best activity against S. typhi (DIZ = 15 mm). The results for both L. camara leaves and stem extracts were analyzed, and both showed a significant difference among the extracts with P-values < 0.05. The lowest MIC against S. aureus 43300, S. typhi, and P. aeruginosa was 50 mg/ml. In search for a synergetic effect between paracetamol and L. camara extracts, positive results were viewed against E. coli, S. typhi B69, and S. typhi

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B71. The important antibacterial activity seen for L. camara can be ascribed to the occurrence of various phytochemicals. The plant can therefore be utilized to treat various diseases caused by the studied pathogenic bacteria. Toxicity and cost-effectiveness studies have to be done before using L. camara extracts as a medicine.

***Keywords*---**antimicrobial resistance, phytosubstances, Lantana camara, inhibition zone, clinical isolated bacteria.

Introduction

Medicinal plants have been used to relieve illness for over 60,000 years ago, maybe even older. Scripts about medicinal plants from ancient civilizations like Egypt and China show people always sought to use nature to cure their diseases. According to WHO, most of the population worldwide (80%) utilize herbal substances to cure diseases. Plants contain phytochemicals that are helpful in the defense against herbivores. These phytochemicals have medicinal properties that are used by humans (Subramanian, 2014). *Lantana camara* (Umuhengeri), an ornamental weed garden plant that belongs to the Verbenaceae family, is a low erect, rugged hairy, evergreen shrub native to tropical America. It is considered as a valuable plant for traditional healers worldwide (Manish *et al.,* 2011). *L. camara* extract has been shown to exhibit antimicrobial, insecticidal, and nematocidal activities. Besides, it exhibits immunosuppressive and antitumor activities (Saraf *et al.,* 2011).

Throughout history, bacterial infections have plagued men. Antibiotics have changed the world in the past 70 years, saving and improving lives and making them the cornerstones of modern health systems. The therapeutic capacity for treating patients with bacterial infections is being depleted by antimicrobial resistance. Physicians encounter infections that are susceptible to few or even none of the available antibiotics (Edward, 2019). Drug-resistant pathogens have made widespread infections more challenging to treat or even incurable in some cases, with catastrophic implications for patients. This was due to antibiotic misuse, incorrect dosage, etc. (Nuha, 2019). In time, as many bacteria are developing antibiotic resistance to conventional antibiotics, natural products from plants are as alternative new antibiotics to cure diseases (Jamshidi-Kia *et al.,* 2018).

The emergence of multidrug-resistant pathogens (MDRPs) forced the development of new antibiotics. However, in developing countries, these new antimicrobial agents' limited availability and affordability remain a significant obstacle to successful MDRP infection treatment (Ntirenganya *et al.,* 2015). As antimicrobial resistance continues to rise, there is currently a scarcity of effective treatments, a general lack of effective preventive measures, and just a few new antibiotics, requiring the advancement of innovative treatment methods and alternating antimicrobial therapies (Marianne, 2017). Over time, the observational efforts of scientists have progressively developed the modern medical system. However, traditional medicine and treatments continue to be the foundation for development. For example, findings in a recent study on *Bixa orellana* L leaves reported antibacterial influence towards *P. aeruginosa*, *S. typhi*, *S. aureus* and *E. coli.* Alkaloids, tannins, saponins, flavonoids, and phenols were the phytochemicals found in *Bixa orellana* L extracts (Manoranjitham *et al.,* 2020).

Plant-based medicines, which were previously only available in the form of crude medicines (teas, poultices, powders), are now used to develop new drug discoveries. Since plants contain various bioactive compounds such as alkaloids, flavonoids, and saponins, they can serve as antimicrobial agents (Sahoo *et al.,* 2013). Bacteria like *P. aeruginosa, S. pneumonia*, *S. aureus, S. typhi* and *E. coli* are the most problematic in Rwanda (Ntirenganya *et al.,* 2015). The development of antimicrobial resistance in patients to available antimicrobials is high and worries some. It is necessary to search for new antimicrobial agents. In search of a new alternative antibiotic drug that may help control drug-resistant pathogenic bacteria, this research focused on the effect of *L. camara* extracts on clinically isolated bacteria (*S. typhi, S. aureus, S. pneumonia, E. coli* and *P. aeruginosa*).

Materials and Methods

Chemicals, plant, and microorganisms

Reagents like extraction solvents (methanol and petroleum ether) and powdered culture media were purchased from Sigma and Himedia wholesale pharmacies. The plant material (stem and leaves) of *L. camara* was gathered from INES-Ruhengeri botanical garden. Clinical bacteria used were *S. aureus, S .typhi, S. pneumonia, P. aeruginosa*, and *E. coli.* They were obtained from Rwanda biomedical center.

Preparation of *L. camara* extracts

The leaves and stems were washed in running water and then dried at 25 °C for 2 successive weeks in the shaded area of the chemistry lab. The dry plant materials were heated in the oven for 30 min at 100 °C and grounded in a fine powder. Three extraction solvents (95% petroleum ether, 95% methanol, and distilled water) were used on both the leaves and stem powder. The maceration technique was followed. 20 g of the fine powder of each plant material was dissolved in 200 ml of each extraction solvent. The beakers were stood on a rotation with frequent agitation for 3 days. The extracts were filtered through nets, and a rotary evaporator was utilized to get the solvents (Laborota 4010 digital). The remnants collected after evaporation was considered crude extracts (Umuhoza *et al.*, 2021; Habyarimana *et al.*, 2022).

Qualitative analysis of phytosubstances

Raaman (2006) method was followed to check the existence of flavonoids, tannins, phenols, alkaloids, glycosides, and saponins. The phytochemicals from water, petroleum ether, and methanol extracts were individually analyzed with two negative controls, viz. sample, and reagent.

Antimicrobial activity and minimum inhibitory level of *L. camara* extracts

Agar well diffusion method was utilized. Clinical isolated bacteria used were first sub-cultured. The Mueller-Hinton agar (MHA) plates were prepared and swabbed differently with pathogenic bacteria. The holes made in the plates with punch borers were seeded with 50 µl plant extracts (100 mg/ml) and allowed to stand for one hour time period. After a proper diffusion, the plates were incubated overnight at 37 °C for 24 h, and then zones of inhibition were noted in mm. The *L. camara* extracts that displayed important antibacterial activity against the pathogens of interest were chosen for the minimum inhibitory level studies. The concentrations considered were 25, 50, and 75 mg/ml *L. camara* extracts. The procedure and incubation were carried as described above.

Synergism between methanol plant extract and paracetamol

5 g of paracetamol tablet was grounded and dissolved in 50 ml of methanol (95%) and water. After spreading the MHA plates with different pathogenic bacteria, equal volumes of methanol plant extract and paracetamol solution were added to the created holes. The incubation of culture plates and inhibition zones measurement were done as described earlier.

Statistical analysis

All experiments were performed in duplicate. The data collected were subjected to two-way ANOVA, and P-values under 0.05 were considered significant. All these analyses were automatically calculated using SPSS.

Results and Discussion

Phytocompound constituents of *L. camara*

Medicinal plants possess various bioactive secondary metabolites responsible for various antimicrobial properties like antibacterial and antifungal (Miyasaki *et al.,* 2020). In the present assessment, alkaloids, glycosides, phenols, flavonoids, saponins, and tannins were noticed in *L. camara* extracts. Phenol and saponins were concentrated in methanol and water extracts (Table 1). These results agree with earlier phytochemical reports on *L. camara* (Naz & Bano, 2013; Salada *et al.,* 2015). Findings in a previous study by Faustin (2012) reported the presence of alkaloids in *L. camara* methanol extracts, which is in contradiction with the present study that indicated the absence of alkaloids in methanol extracts. The difference in biochemical composition of the same plant may be attributed to the different factors like geographic location or the development stage of the plants used, as suggested by different studies (Randrianalijaona *et al.,* 2005; Ganjewala *et al.,* 2009). The presence of active substances like tannins, flavonoids, and phenols shows *L. camara* may have great pharmacological potential against microorganisms (Nayak *et al.,* 2008; Ganjewala *et al.,* 2009; Faustin, 2012; Kalita *et al.,* 2012; Naz & Bano, 2013; Salada *et al.,* 2015; Kasithevar *et al.,* 2017; Al-Snafi, 2019; Claude, 2019; Shaikh *et al.*, 2022).

Table 1
Phytochemicals screened from *L. camara* extracts. +: presence –: absence

Phytochemicals	Leaves			Stem		
	Methanol	Petroleum ether	Water	Methanol	Petroleum ether	Water
Alkaloids	-	+	+	-	+	+
Flavonoid	+	+	-	+	+	+
Glycosides	+	+	+	+	+	+
Phenolics	+	-	+	+	-	+
Saponins	+	-	+	+	-	+
Tannins	+	-	+	+	+	-

Antimicrobial activities of *L. camara* stem and leaf extracts

Plants are currently taken as a new vital source of phytocompounds that could be used in the creation of new therapeutics. They also exhibit fewer side effects compared to synthetic ones (Iwu *et al.,* 1999). This study investigated *L. camara* leaf, and stem extracts against *S. typhi, S. aureus, S. pneumonia*, *E. coli* and *P. aeruginosa* were investigated. The *L. camara* extracts were active against investigated pathogenic bacteria. *L. camara* methanol leaf extract repressed optimally *S. aureus* 43300 growth (DIZ = 28 mm), followed by *P. aeruginosa*, and *S. typhi*. Among the *L. camara* stem extracts, methanol extract was the most efficient. In contrast, water stem extract didn't show any activity against any bacterium, and petroleum ether stem extract was only effective against *S. aureus* 43300 (Table 2). The results on methanol leaf extract against *P. aeruginosa* and *S. aureus* 29213 are similar to findings by Naz and Bano (2013), as they reported inhibition zones of 21 mm for *S. aureus* and 20 mm for *P. aeruginosa*.

The results for both *L. camara* leaves and stem extracts were analyzed using two-way analysis of variance (Table 3 & Table 4), and both showed significant differences within the extracts with P-values < 0.05. These values show that the solvents used in the extraction contributed to the efficacy of the extracts. Comparing the means, *L. camara* methanol extracts were most efficient. In that regard, it has been viewed in many studies that alcoholic extracts are more efficient (Nayak *et al.,* 2008; Badakhshan *et al.,* 2009; Faustin, 2012; Kalita *et al.,* 2012; Naz & Bano, 2013; Salada *et al.,* 2015; Kasithevar *et al.,* 2017; Al-Snafi, 2019). Traditional healers have long used lantana species, particularly *L. camara*, to treat a variety of human maladies such as gastrointestinal and dermatological infections, tetanus, and malaria (Badakhshan *et al.,* 2009), and the presence of antibacterial activity demonstrated in the current study proved *L. camara* extracts can be a source of treatment for infections such as typhoid caused by *S. typhi*, urinary tract infections caused by *P. aeruginosa*, and wound infections caused by *S. aureus*. Antibacterial activity of *L. camara* could be ascribed to various phytosubstances present in a significant amount (Kasithevar *et al.,* 2017).

Table 2
Means of zones of inhibition (mm) of *L. camara* extracts against pathogenic bacteria. –: resistance

Bacterium	Leaves extract			Stem extract		
	Petroleum ether	Water	Methanol	Petroleum ether	Water	Methanol
S. aureus 29213	13.0 ± 0.8	17.0 ± 1.1	20.5 ± 0.8	-	-	8.0 ± 0.2
S. aureus 43300	17.0 ± 0.6	-	28.0 ± 1.5	10 ± 0.5	-	-
P. aeruginosa	-	-	24.5 ± 1.3	-	-	14.0 ± 0.7
E. coli 35218	15.0 ± 1.1	-	15.0 ± 0.8	-	-	14.0 ± 0.6
S. typi B69	21.0 ± 1.3	15 ± 0.9	23.0 ± 1.1	-	-	15.0 ± 1.0
S. typi B71	12.0 ± 0.8	-	23.5 ± 1.4	-	-	-
S. pneumonia	19.0 ± 0.9	-	23.0 ± 0.9	-	-	-

Table 3
Two-way assessment of variance of the *L. camara* leaves extracts

Variation source	Sum of squared	Degree of freedom	Mean square	Ratio of two mean square	P-value	F critical
Bacterial species	284.9048	6	47.48413	1.228921	0.357218	2.99612
Solvents	1125.5	2	562.75	14.56434	0.000617	3.885294
Error	463.6667	12	38.63889			
Total	1874.071	20				

Table 4
Two-way investigation of variance of the *L. camara* stem extracts

Variation source	Sum of squared	Degree of freedom	Mean square	Ratio of two mean square	P-value	F critical
Bacteria	83.14286	6	13.85714	0.532967	0.773561	2.99612
Solvents	208.6667	2	104.3333	4.012821	0.046299	3.885294
Error	312	12	26			
Total	603.8095	20				

Screening for the minimum inhibitory concentration

Common patient infections are not treated easily owing to pathogens that are resistant to various drugs. MIC values may be utilized for the identification and evaluation of the activity of phytosubstances against bacteria-causing diseases (Wiegand *et al.,* 2008). *L. camara* extracts that showed the highest antimicrobial activity were diluted to investigate their extent of activity. MIC was investigated by dilution of methanol leaves extracts using distilled water as they showed the highest inhibition zones. The lowest MIC was 50 mg/ml that showed activity against *S. aureus* 43300, *S. typhi*, and *P. aeruginosa*. Table 5 shows the MIC of *L. camara* methanol leaves extracts.

Table 5
Minimum inhibitory concentration of *L. camara* methanol leaves extracts. -: resistance

Bacteria	Leaves extract Methanol		
	100 mg/ml	75 mg/ml	50 mg/ml
S. typi B69	23.0 ± 1.6	10.0 ± 0.6	3.0 ± 0.1
S. aureus 43300	28.0 ± 1.5	15.0 ± 1.0	6.0 ± 0.2
P. aeruginosa	24.5 ± 0.9	11.0 ± 0.8	4.0 ± 0.3
S. typi B71	23.5 ± 1.2	13.0 ± 1.1	-
S. pneumonia	23.0 ± 0.8	9.0 ± 0.4	-

Synergism between *L. camara* extracts and paracetamol

Antibiotic resistance is globally becoming a more serious clinical problem. Although pathogenic bacteria are tolerating current antibiotic groups, few studies are being conducted to find new alternative solutions. Antibiotic combinations are often used to extend the antimicrobial spectrum of each other and achieve synergistic results (Jouda *et al.,* 2016). In screening for synergistic effect between paracetamol and *L. camara* extracts, activity was only viewed in methanol extracts (Table 6). Methanol leaves extracts were more effective with paracetamol against *E. coli*, *S. typhi* B69 (DIZ = 26 mm), and *S. typhi* B71. In disparity, methanol stem extracts showed synergy with paracetamol against *S. aureus* 43300 and *S. typhi.* El-Bashiti *et al.* (2016) reported similar synergetic effect against *E. coli* when *L. camara* extract and paracetamol were combined.

Table 6
Synergism between *L. camara* methanol leaf extracts and paracetamol. –: means resistance, M: methanol, P: paracetamol

Bacteria	Leaves extracts		Stem extracts	
	M (25 µL)	M (25 µL) + P (25 µL)	M (25 µL)	M (25 µL) +P (25 µL)
S. aureus 29213	17.0 ± 1.2	19.0 ± 1.2	8.0 ± 0.4	-
S. aureus 43300	20.0 ± 1.4	14.0 ± 0.8	-	11.0 ± 0.9
P. aeruginosa	17.0 ± 0.9	14.0 ± 1.0	14.0 ± 1.0	14.0 ± 1.1
E. coli 35218	10.0 ± 0.9	22.0 ± 1.1	14.0 ± 0.7	0 ± 0.0
S. typi B69	19.0 ± 1.3	26.0 ± 1.3	15.0 ± 0.8	17.0 ± 0.8
S. typi B71	19.0 ± 1.1	24.0 ± 1.4	-	10.0 ± 0.5

Conclusion

The present study indicated that *L. camara* leaves and stems possess antibacterial activity that may be credited to the presence of different phytochemicals like tannins, glycosides, alkaloids, flavonoids, phenolics, and saponins. Methanol leaf extracts of *L. camara* showed a greater potential than others. Furthermore, it exhibited a synergetic activity with paracetamol against bacteria like *E. coli* and *S. typhi.* Based on this study's findings, *L. camara* extracts have to be further investigated to isolate the individual phytochemicals present. Other plant elements such as roots and flowers should be studied for their usefulness. To assess the safety indices of the extracts, toxicity tests of *L. camara* should be carried out. Clinical trials should be conducted to investigate the potential of *L. camara* extracts in treating infectious diseases caused by the studied bacteria.

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