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A Case of Herbal Antimicrobial Chemotherapy of Polymicrobial Infections and Diseases: Preliminary Evaluation and Efficacy of Antimicrobial Activities of *Lantana camara* Leaf Extracts Against Gastrointestinal Tract and Skin Infections Pathogens

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Abstract- ***Lantana camara is a medicinal plant used traditionally to treat infections and diseases. This study investigated the phytochemical constituents and antimicrobial potential of its leaf extracts against clinical pathogens causing gastrointenstinal and skin infections. The leaves were extracted using ethanol, methanol, aqueous solvents and was subjected to qualitative phytochemical screening. The antimicrobial activities of lantana camara leaf extracts was evaluated against some bacterial isolates such as Gram positive: Staphylococcus aureus Staphylococcus epidermidis, and Gram negative: Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Salmonella typhi-II, Proetus mirabis, Salmonella dysentrariae, Shigella spp and some clincally selected fungal isolates: Candida albicans, A.niger, by the agar ditch diffusion method. All the extracts, exhibited excellent antimicrobial activity against the tested microbial isolate with inhibition zone within the range of 25.0 - 38.0 mm, in the descending order: methanol > ethanol > aqueous, with minimum inhibitory concentration (MIC) values ranging between 62.5 - 250.0 mg/ml and minimum biocidal concentration (MBC) value of 1000.0 mg/ml, indicating time-dependent biostatic activity.These findings suggests that L. camara leaf extract possesses bioactive compounds with potential antimicrobial properties, supporting its ethnomedicinal usages in the herbal treatment and control of infections caused by the pathogens.***

Keywords: Herbal Antimicrobial chemotherapy, polymicrobial infections and diseases, Preliminary evaluation and efficacy, antimicrobial activities, Lantana camara, leaf extracts, gastrointestinal tract, skin infections pathogens.

I. INTRODUCTION

The use of plants in the development of drug dosage forms for the treatment and management of infections and diseases dates back to history. The last few decades have witnessed a renewed interest in plants as alternative antimicrobial and therapeutic agents, due to increasing microbial resistance to the available antibiotics. These plants parts owe their therapeutic effects to different secondary metabolites with diverse mechanisms of action. Among these plants, *Lantana camara* Linn, a hardy colourful shrub often dismissed as weed holds surprising promise. The plant belongs to the Family Verbenaceae, commonly known as wild or red sage and locally: Hausa (Kimbamahalba); Yoruba (Ewonadele); Igbo (Anya nnunu) and Efik/ Ibibio (Lantanna). *Lantana camara* is the most widespread species of this genus and regarded both as a notorious weed and a popular ornamental garden plant (Ganjewala *et al.,* 2009). *L. camara* extracts are used in herbal medicine for the treatment of various human infections and diseases such as skin itches, boils and abscesses, ringworm, athlete foot, dermatitis, eczema, dysentery and diarrhoea, intestinal worms, stomach ulcers and other conditions such as leprosy, chicken pox, measles, asthma, ulcers, tumors, an has remarkable effects on high blood pressure, tetanus and rheumatism etc. (Barre , *et al,* 1997). Extracts from the leaves have been reported to have antimicrobial: mostly, antifungal, insecticidal and nematicidal activities

(Anderson, 2003). Furthermore, the essential oils extracted from *L. camara* play an essential role in medicine, in addition to their tremendous value in the cosmetics and nutraceutical industries. The use of essential oils of aromatic plants for food and medicinal purposes have been a focus of research in health sciences, due to their varied biological importance and activities such as antibacterial, analgesic, anti-inflammatory, anti-parasitic, antioxidant, and antitumor activity (Sousa *et al*, 2010; Jaradat *et al* 2020). The oils differ in their chemical compositions according to geographic origins of the plants. *L. camara* essential oil containing β-caryophyllene, geranyl acetate, Terpinyl acetate, bornyl acetate and limonene remarkedly inhibited the growth of many tested bacteria and fungi such as *P. aeruginosa, A. niger, C. albicans*, which appeared to be the most sensitive ones. (Da Silva *et al*. 1999; Deena and Thoppil, 2000 Sefidkon, 2002; Kasali *et al*. 2004).

This study was carried out to ascertain and justify the folkloric claim of antimicrobial activity of *L. camara* extracts against notably gastrointestinal tract and skin infectious pathogens, to further ascertaining and validate the claims of its antimicrobial activity potency and used in the treatment of polymicrobial infections and diseases, particularly dysentery and diarrhea.

II. MATERIALS AND METHODS

Plant Collection and Identification

Fresh leaves of *Lantana camara* used in this study were collected from Uyo Akwa Ibom State, Nigeria, in July 2024. The desired part of the plant (leaf) were cut with scissors and put in polythene bag to minimize moisture loss. The collected samples were then transported and stored, in air-tight poly ethene bags to minimize moisture loss.

Plant preparation

The collected leaves were chopped into smaller pieces, rinsed with distilled water to remove any impurities such as sand and debris, and then air- dried at room temperature in the laboratory for two weeks. Once completely dried, the samples were pulverized into fine powder using a mortar and pestle. The powdered leaf samples were stored in airtight containers to preserve their active ingredients before extraction.

Extraction of Plant Materials

Extraction was done following the standard phytochemical procedures, using aqueous, ethanol and methanol solvents (Trease and Evans, 2002). For each solvent, 500.0 g of the pulverized plant part (leaf) was cold- macerated in 500.0 ml of distilled water for 24 hours; and in 75 % 500.0 ml of ethanol and methanol respectively for 72 hours in 1000.0 ml conical flask with intermittently stirring. In each case, the mixture was filtered thrice through a funnel, fitted with cotton wool and filter paper. The respective filtrates were concentrated *in vacuo* in a water bath at 50 ^{o}C for 5 days and their respective yields were obtained.

Qualitative Phytochemical Screening

Qualitative phytochemical analysis were performed following standard phytochemical procedures on the leaf extracts to identify potentially bioactive phytochemicals present in the extracts (Trease *et al.,*2002).

Collection, Characterization and Maintenance of Test Organisms

Ten clinically significant test organisms for the antimicrobial assays were obtained from the University of Uyo Medical Center (UUMC) and University of Uyo Teaching Hospital (UUTH). The isolates included Gram- positive bacteria *Staphylococcus aureus and Staphylococcus epidermidis,* Gram- negative bacteria: *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Proteus spp, Salmonella epidemic, Shigella spp.*and two fungal isolates *Candida albicans, Aspergillus niger.* The bacterial isolates were cultured on nutrient agar at 37 ^{o}C for 24 hours, while the fungal isolates were grown on Sabouraud dextrose agar at 28 ^{o}C for 48 hours. These organisms were subcultured to obtain pure cultures and identified using standard microbiological and biochemical procedures (Tilton and Howard, 1987; Baron and Finegold,1990; Ekong *et al* 2004, Obioha *et al* 2023).

Standardization of Test Organism

Freshly cultured organism 18 – 24 h at 37 ^{o}C (bacteria) and 24 – 48 h at 28 ^{o}C (fungi) were tenfold serially diluted in both nutrient - broth, NB (bacteria) and Sabouraud dextrose broth, SDB (fungi). The turbidity of the various suspensions were standardized to a 0.5 McFarland Nephelometer standard, with an estimated cell density of $1x10^{8}$ cfu/mL (Tilton and Howard, 1987; Baron and Finegold,1990; Ekong *et*

al 2004). The Gram- positive and fungal cultures were adjusted to factor 3; while the Gram- negative cultures, were adjusted to factor 5 respectively (Ekong *et al*, 2004).

The purity of each standardized microbial inoculum was confirmed by spread-plating 0.1 ml of each diluted suspension onto the appropriate culture media to check for contamination or overgrowth (Ekong *et al*., 2004).

Evaluation of Antimicrobial Sensitivity Test of Test Organism to *Lantana camara* Leaf Extracts

The antimicrobial activities of *Lantana camara* leaf extracts were evaluated against the standardized test organism using agar ditch diffusion method (Ekong *et al*., 2004). A sterile spatula was used to create a ditch to avoid damage to the agar surface. The extracts were weighed and dissolved using sterile water to create the different concentrations (1000.0 mg/ml; 500.0 mg/ml, 400.0 mg/ml, 300.0 mg/ml, 200.0 mg/ml, 100.0 mg/ml). Out of each concentration of the extracts, 0.5 ml was used to fill the ditch made on the nutrient agar (NA) and Sabouraud Dextrose Agar (SDA) plates respectively. Thereafter, each microbial culture was streaked away from the *L. camara* leaf extracts in the ditch in the nutrient agar NA (bacteria) and SDA (fungi) in parallel and alternate lines from each other. All plates were allowed to stand for one hour for pre- diffusion of the extracts into the media. All plates were incubated at 37 ^{0}C for bacteria and at 28 ^{0}C for 48 hours for fungi. For all the isolates, antimicrobial activity was determined by measuring the diameter of zone of growth inhibition in millimeters, for each of the cultures, in triplicates.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Biocidal Concentration (MBC).

The MIC of *L. camara* leaf extracts against the test isolates was determined by two-fold serial dilution of the concentrations in nutrient broth (NB) and Sabouraud dextrose broth (SDB) following the macro- broth dilutions technique (Tilton and Howard, 1987; Baron and Finegold, 1990, Ekong *et al*, 2004;). The respective two- fold serially diluted graded concentrations of the various *L. camara* extracts, were inoculated with aliquots of the standardized microbial inocula and incubated under standard conditions for both bacteria and fungi. For each extracts, the least or lowest concentration that inhibit the growth of the test organisms was taken as the MICs, of the extracts against the test organisms. Similarly, the MBC of the *L. camara* extracts against the test organisms was carried out following the macro-broth technique of the MIC. This was determined by re- incubating the non MIC test tubes further for 48 hours. The least or lowest concentration that did not permit the growth of the organism after 48 hours of incubation at 37 ºC (bacteria) and 28 ºC (fungi), was regarded as the minimum biocidal concentrations (MBCs), of the extracts against the organisms.

III. RESULTS AND DISCUSSION

The results of the phytochemical screening of *Lantana camara* leaf extracts are presented in Table 1

Table 1 Phytochemical Screening of *L. camara* leaf Extracts

S/N	Test	Aqueous extract	Ethanol extract	Methanol extract
1	Alkaloids	+	+	+
2	Saponins	+	+	+
3	Tannins	+	+	+
4	Anthraquinone	+	+	+
5	Cardiac glycosides	+	+	+
6	Flavonoids	+	+	+
7	Phenols	+	+	+
8	Terpenes	+	+	+

Key: + = Present - = Absent

Medicinal plants constitute an important source of bio-active compounds because of the phytochemical diversity found in several species. Thus, from time immemorial and in recent decades, certain plants

have been evaluated for their antimicrobial activities worldwide (L.Sagar *et al*, 2005) Various extracts of medicinal plants have been reported to show antimicrobial activities, due to the abundant presence of phytochemicals. In this study, *L. camara* leaf extracts have showed the abundant presence of phytochemicals such as: alkaloids, saponins, tannins, anthraquinone, cardiac glycosides, flavonoids, phenols and terpenes. These phytochemicals and others present in the various extracts of medicinal plants including *L. camara* have been reported to enhance and be responsible for the observed antimicrobial activities against various clinical pathogens of the skin, polymicrobial gastrointestinal tract (GIT); viral and helminthic infections and diseases.

The result of the antimicrobial sensitivity test of the test organisms, against *L. camara* leaf, aqueous, ethanol and methanol extracts is presented in Table 2.

Table 2: Antimicrobial Activities of *Lantana camara* leaf extracts

Test Organisms	Antimicrobial Agents (mg/mL)/Inhibition Zone Diameter (mm)				
	Extract Concentrations (1000mg/mL)			Standard Antibiotics/Control (mg/ml)	
	Aqueous	Ethanol	Methanol	Chloramphenicol	Ketoconazole
S. aureus	28.0	30.0	37.0	23.0	NT
S. epidermidis	25.0	25.0	30.0	18.0	NT
S. typhi	38.0	25.0	38.0	20.0	NT
S. typhi – 11	30.0	35.0	35.0	19.0	NT
P. mirabilis	35.0	28.0	35.0	18.0	NT
P. aeruginosa	22.0	36.0	35.0	18.0	NT
E. coli	30.0	35.0	31.0	20.0	NT
S. dysentrariae	38.0	23.0	35.0	26.0	NT
C. albicans	-	35.0	33.0	NT	18.0
A. niger	-	28.0	30.0	NT	16.0

Key: NT = Not Tested – = No Activity

All the leaf extracts of *L. camara* exhibited excellent broad-spectrum of antimicrobial activities against the bacterial and fungal isolates tested with excellent inhibition zones within the range 22.0 - 38.0 mm except aqueous extract, with no antifungal activity, 18.0 – 26.0 mm (bacteria) and 16.0 – 18.0 mm (fungi) compared to the control antibiotics, the result indicated that *L. camara* leaf extracts possess excellent/ potent antibacterial and antifungal activities, specifically, ethanol and methanol extracts exhibiting antifungal activity; while the aqueous extract did not possess antifungal activity. Thus, the antimicrobial potency of the extracts were in the descending order: methanol > ethanol > aqueous. The excellent performance and potency of *L. camara* leaf extracts against the wide array of microorganisms tested, being a function of the abundant presence of the indicated phyto-constituents, informed the folkloric uses of this plant extracts, as an effective and potent antimicrobial agent in several polymicrobial infections and diseases. Many studies have been conducted on the antibacterial activities of *L. camara* (Sonibarre,2008). Thus, a follow- up effort, this study showed that various concentrations of *Lantana camara* extract were active against various clinical pathogens of the skin and gastrointestinal tract. Furthermore, *L. camara* have been widely reported to possess a broad spectrum of antimicrobial activities (Tesch, 2011). This was confirmed as the microorganisms used in this study were found to be susceptible to the leaf extracts of *Lantana camara,* suggesting that the antimicrobial principle contained in these plant parts may be of broad spectrum since they were able to inhibit both Gram-positive and Gram-negative bacteria as well as fungi. Sharma *et al* (2013), also made similar observations. This validates its use as an antimicrobial agent in the treatment of numerous polymicrobial infections and diseases, as well as conditions caused by microbial infections. Presumably, it was earlier mentioned in the literature review that the antimicrobial activity can be attributed to the presence of phytochemicals present in the plant such as terpenes and flavonoid compounds (Misra *et al*. 2000) which have been employed as a disinfectant and remain the bench mark for comparing other bactericides. Alkaloids, saponins and terpenes also play a major role in its antimicrobial activity.

These organisms, because of their low intrinsic susceptibility to antimicrobial agents and the rate with which they acquire resistance horizontally, are proving to be especially troublesome within the hospital environment, resulting in frequent nosocomial infections (Okeke *et al*., 2016). As these organisms are uniformly susceptible to the extracts of *L. camara in vitro*, suggests the possibility of using the plant as a source of active antimicrobial principles against infections caused by the susceptible organisms. The differences in the susceptibilities of the isolates to the plant extracts can be related to the constitutional or structural variability of the tested organisms as that cell wall composition of the organisms differ. However, this study is inconclusive of which leaves extract is more promising in terms of antimicrobial activity, even though a study by Gatsing, *et al*. (2010), reported that the ethanol leaf extract exhibited the highest antibacterial activity in comparison to methanol, ethanol, ethyl acetate and acetone extracts of *L. camara* leaves indicating that the active principle could be very polar in nature.

The findings from this research on the antimicrobial activities of the extracts from *L.camara* have further strengthened the previous findings on the effectiveness of extracts of this medicinal plant against polymicrobial infections. The activity of the standard drugs was also compared to the activity of the extracts and it was found that the standard drugs has more activity compared to the extract. These results are in line with Okeke *et al*. study (2019) who showed that ethanolic extracts of *Lantana camara* leaves exhibited antibacterial activity against these strains. Other researchers confirm these results which are similar to our findings (Okeke *et al*. 2019). This could be explained by the fact that the active compounds available in the ethanolic extract have less affinity with water. On the other hand, the resistance genes would have strengthened the defensive arsenal of the bacteria, making them more difficult to eliminate. These results would be due to the phytochemical composition of the *L. camara* leaf extract.

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Biocdial Concentration (MBC) of *L. camara* leaf extracts against Test Organisms.

Test Organisms	MIC Extracts (mg/mL)			MBC Extracts (mg/ml)			MIC Standard (mg/ml)		MBC Standard (mg/mL)	
	Aqueous (AQ)	Ethanol (ET)	Methanol (ME)	Aqueous (AQ)	Ethanol (ET)	Methanol (ME)	Chloramphenicol (CHC)	Ketoconazole (KTZ)	Chloramphenicol (CHC)	Ketoconazole (KTZ))
S. aureus	125.0	125.0	125.0	1000.0	1000.0	1000.0	23.0	NT	23.0	NT
S. epidermidis	125.0	125.0	125.0	1000.0	1000.0	1000.0	18.0	NT	18.0	NT
S. typhi	125.0	250.0	65.5	1000.0	1000.0	1000.0	20.0	NT	20.0	NT
S. typhi – 11	125.0	250.0	65.5	1000.0	1000.0	1000.0	19.0	NT	19.0	NT
P. mirabilis	250.0	125.0	65.5	1000.0	1000.0	1000.0	18.0	NT	18.0	NT
P. aeruginosa	125.0	250.0	125.0	1000.0	1000.0	1000.0	18.0	NT	18.0	NT
E. coli	125.0	250.0	125.0	1000.0	1000.0	1000.0	20.0	NT	20.0	NT
Sh. dysentrariae	125.0	125.0	65.5	1000.0	1000.0	1000.0	26.0	NT	26.0	NT
C. albicans	125.0	125.0	125.0	1000.0	1000.0	1000.0	NT	18.0	NT	18.0
A. niger	125.0	250.0	250.0	1000.0	1000.0	1000.0	NT	16.0	NT	16.0

Key:-AQ = Aqueous Extract; ET = Ethanol; ME = Methanol Extract; CHC = Chloramphenicol; KTZ = Ketoconazole; NT = Not Tested

The results of minimum inhibitory concentrations (MICs) and minimum biocidal concentration (MBC) of *Lantana camara* leaf extracts against the test organisms is presented in Table 3. The extracts recorded relatively low and appreciable MIC values within the range of (62.5 – 250.0 mg/ml) for both bacterial and fungal isolates tested; as well as the uniformly elevated MBC value of 1000.0 mg/ml for all the microbial isolates. The generally low MIC values of the extract confirms the potency of *L. camara* as an effective herbal remedy for various usage as antimicrobial agents in the folkloric treatment of wide array of polymicrobial infections and diseases. Furthermore, the uniformly elevated MBC value of the extracts for all the test organisms is an indication of killing rate at other higher concentration over time. Thus the MIC and MBC values and the corresponding MIC: MBC indices for the test organisms; suggest a cidal mode of activity for the *L. camara,* extracts which is basically concentration - dependent. According to the MIC and MBC values, there is a variation in the level of activity between the pathogens and the extracts, which increases with extract concentration over time. The effect of ethanol leaf extract on the microbial strains showed, that the extract is fungicidal to *Candida albicans* and *Aspergillus niger* and also bactericidal to *Staphylococcus aureus*, *Staphylococcus epidermitis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella*

spp, *Proteus mirabis.* Hence, these findings suggest that all *L. camara* extracts used in this study, may be considered bactericidal or fungicidal and depending on the higher concentration over time. Hence, the extracts of *L. camara,* could be asserted to exhibit a broad- spectrum concentration - dependent bactericidal and fungicidal activities against the test organisms.

In conclusion, from this study, the phytochemical analysis revealed the presence terpenoids, flavonoids, saponins, anthraquinones and phenolic acids suggesting that these compounds can contribute to observed excellent antimicrobial activities and the continued and bactericidal and fungicidal effects. This study demonstrates that *L. camara* leaf extracts exhibit excellently significant broad spectrum concentration- dependent biocidal antimicrobial activity against two Gram-positive bacteria, six Gram-negative bacteria and two fungal isolates. Which are the clinically relevant pathogens of the skin and gastrointestinal tract including: *Staphylococcus aureus*, *Staphylococcus epidermitis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella spp*, *Proteus mirabis* and *Candida albicans* and *Aspergillus niger* tested. The results showed that ethanol and methanol extracts of the *L. camara* leaves possess sterling broad spectrum antimicrobial activities, while the aqeous extract, did not possess antifungal activity against the selected test organisms. The differences in the activity of the extracts have been ascribed to the differences in solubility of the active ingredient in each solvent. Thus, plant constitutes a potential antimicrobial activity that can be explored as remedy for the numerous human bacterial and fungal co-infections. Accordingly the results of this study of different solvents and other parts of the plant may provide valuable information for further detailed studies of active compounds, necessary for the development of antimicrobial chemotherapeutic agents in the future as the plant offers wide-scope for utilization as lead raw materials by pharmaceutical industries for drug discovery and development/ formulation.

From the findings of the study, the following recommendations are made:
Comprehensive research into the bioactivity, therapeutic value, and roles played by each of the numerous phytoconstituents of *L. camara* should be carried out; proper standardization of bioactive compounds and comprehensive well-controlled clinical trials should be done to evaluate the efficacy and safety of *L. camara* extracts in pharmaceuticals in order to derive optimum health benefits of this plant, more studies towards the antimicrobial activity *in vivo* as well as the mechanism of action needed to be conducted for better understanding and applications of *L. camara* extracts as potent antimicrobial remedies.

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