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Evaluation of Antibacterial Effects and Phytochemical Analysis of *Lantana camara linn* Leaf and Berry Extracts

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Authors' contributions

This entire work was done with collaborative effort of all authors. Author AAA designed the study and wrote the first draft of the manuscript. Authors OOO and OTF carried out the field sampling, laboratory research and interpretation of laboratory analysis. Author DAA involved in the critical revision of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

In order to assess the antibacterial activities of methanolic and aqueous leaf extracts (MLE and ALE) as well as that of methanolic berry extract (MBE) of *Lantana camara Linn*, we screened each for antibacterial effects against 14 bacterial isolates using agar-well diffusion method at concentration of 25mg/ml with streptomycin as control antibacterial. Data obtained were analyzed with ANOVA and t-test using SPSS 15.0 for Windows. The MLE is comparable to streptomycin in antibacterial activity as they both showed antibacterial activity against 13 (92.86%) bacterial isolates with inhibition zone diameter (IZD) of 12mm-20mm. The ALE and MBE however, showed inhibitory activities against 3 (21.43%, IZD 10mm-11mm) and 7 (42.86%, IZD 11mm-17mm) bacterial isolates respectively. Flavonoids, saponin and alkaloids were present in the three extracts while phlobatannin, cardiac glycoside and steroid were absent. Terpenoids and tannin were only present in the MBE and MLE respectively. Sodium, potassium, calcium, magnesium and zinc were observed in the leaf and berry of Lantana camara. Iron, copper and manganese were present in trace amount with lead (Pb) totally absent. Though the three extracts showed antibacterial effects

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against the isolates, the MLE was the most effective. The phytochemical analysis revealed that *Lantana camara* has compounds with antibacterial activities and can possibly be used as alternative therapy to infections caused by the sensitive study bacteria.

Keywords: Lantana camara Linn; leaf; berry; methanolic extracts; aqueous extracts; antibacterial; phytochemical compounds.

1. INTRODUCTION

Traditional medicine which includes the use of herbs and plant-based products for different therapeutic purposes has been in existence before the inception of modern scientific medicine [1]. Plant-based medications still remain important and most applied therapeutic arsenals for treatment of large number of diseases [2]. Moreover, the diminishing efficacy of synthetic drugs that arise as a result of emergence of different mechanism of antimicrobial resistance exhibited by multidrug-resistant microorganisms has prompted the scientists to search and screen for medicinal plants for their antimicrobial properties.

Plant berries have been reported to be rich in phenolic compounds with interesting biological activities such as antioxidant, anti-carcinogenic, [3-5] and antimicrobial properties against human pathogenic bacteria [6-7]. The antimicrobial and biological activities of many plants have been attributed to the different phytochemical compounds such as alkaloids, glycosides, tannins and saponins present in them [8].

Lantana camara is a species of flowering plant in the family Verbenaceae, native to the tropical and subtropical regions of South America. It is referred to as “Ewon Agogo” in Yoruba language spoken by a tribe in Western part of Nigeria. It is a woody straggling plant, commonly known as red or wild sage with various flower colors, leaves with rounded tooth edges which comprise 650 species.

Lantana camara is a noxious ornamental weed that has found its application as antitumour, antifungal, antimalarial and analgesic agents. It has also been used in treatment of signs of respiratory infection conditions such as cough, cold, asthma and bronchitis [9-12]. In addition, wound healing properties of *Lantana camara* has been reported [8].

Several studies have reported the antimicrobial properties of leaf extracts of Lantana camara [13-17]. However, there is paucity of literature on the antibacterial properties of berry extracts of *Lantana camara* in Nigeria. This study therefore qualitatively analyzed the phytochemical constituents and investigated the antibacterial properties of methanolic and aqueous leaf as well as methanolic berry extracts of *Lantana camara.*

2. MATERIALS AND METHODS

2.1 Collection of Samples

Fresh leaves and berries of *Lantana camara* were collected at different areas of Alekuwodo in Osogbo, Osun State, Nigeria. The plant was identified and authenticated at the herbarium unit of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

2.2 Methanolic and Aqueous Extraction of Leaves and Berries of *Lantana camara linn*

The leaves and berries of *Lantana camara Linn* were dried in the hot air oven at 40ºC and ground into fine powder. For the preparation of methanolic extract, about 180g each of the powdered specimens was extracted with methanol/distilled water (3:2) for four days. Distilled water was used for the preparation of aqueous extracts. The extracts were then filtered and the filtrate was concentrated in vacuum using a rotary evaporator. The concentrated extracts were freeze-dried and stored for subsequent qualitative phytochemical analysis and antibacterial testing.

2.3 Test Organisms

Fourteen bacterial isolates were used. The Gram positive bacteria includes *Bacillus polymyxa* (LIO, locally isolated organism; isolated from pus and wound infection), *Micrococcus luteus* (NCIB 196; now NCIMB 196), *Bacillus cereus* (NCIB 6349; now NCIMB 6349), *Clostridium sporogenes* (NCIB 532; now NCIMB 532), *Corynebacterium pyogenes* (LIO), Methicillin resistant *Staphylococcus aureus*, MRSA (LIO), *Bacillus anthracis* (LIO), *Bacillus stearothermophilus* (NCIB 8222; now NCIMB 8222) and *Bacillus substilis* (NCIB 3610; now NCIMB 3610). The Gram negative bacteria were *Escherichia coli* (NCIB 86; now NCIMB 86), *Klebsiella pneumoniae* (NCIB 418; now NCIMB 418), *Pseudomonas aeruginosa* (NCIB 950; now NCIMB 950), *Pseudomonas fluorescence* (NCIB 3756; now NCIMB 3756) and *Proteus vulgaris* (LIO).All bacteria isolates used in this research except the methicillin resistant *S. aureus* which was isolated from pus were obtained from culture collection from Dr. D. A. Akinpelu, of Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

2.4 Antimicrobial Sensitivity Testing of Crude Extracts

The crude extracts were screened for antimicrobial activity using agar well diffusion method as described by Russell and Furr [18]. The various test bacteria were standardized using the 0.5 McFarland turbidity standards and then tested against the effect of the plant extracts at a concentration of 25mg/ml each on Diagnostic sensitivity testing (DST) agar. The plates were later incubated at 37ºC for 24h after which they were observed for zones of inhibition. The inhibition zone diameter was measured in millimeters. The antimicrobial effects were compared with that of the standard antibiotic streptomycin at a concentration of 1mg/ml [19].

2.5 Qualitative Phytochemical Analyses of Leaves and Berries

The phytochemical analysis of the extracts was done using standard procedure as described by Edeoga et al. [20] and Sofowora [21]. The extracts were screened for the following phytochemical compounds: phlobatannis, flavonoids, cardiac glycosides, terpenoids, saponins, steroids and alkaloids.

2.6 Proximate Nutrient Analysis of Leaves and Berries

The proximate analysis of nutrient of leaves and berries were done using standard methods of analysis of Association of Official Analytical Chemists [22]. Parameters estimated are ash content, moisture content, protein, fat, fiber and carbohydrate.

2.7 Mineral Analysis of Leaves and Berries

The mineral analysis of leaves and berries for Na, K, Mg, Ca, Zn, Fe, Cu, Mn, Pb and P was done using atomic absorption spectrophotometric method. The concentration was expressed as part per million (ppm).

2.8 Statistical Analysis

Results were expressed as means ± standard deviation. The data were analyzed using Analysis of variance (ANOVA) and student's t-test with SPSS 15.0 for Windows. P≤0.05 was used as indicator of significant difference.

3. RESULTS AND DISCUSSION

3.1 Antibacterial Activities

The antibacterial activities of the MLE, ALE and MBE at concentration of 25mg/ml (Table 1) revealed that MLE was comparable to the control (streptomycin) as they both showed inhibitory activities against thirteen (92.86%) out of the fourteen bacterial isolates with IZD ranging from 10 to 20mm and 11mm to 24mm respectively. The ALE was active against only three (21.43%) while the MBE was active against six (42.86%) bacterial isolates with IZD ranging from 10 mm to 11mm and 11mm to 17mm respectively. The antibacterial result suggested that methanol could be a preferable agent for extraction of active ingredients of plant parts such as leaves, berry stem and roots [15,16,23]. *P. vulgaris* exhibited the lowest IZD (10mm) while the MRSA exhibited the highest IZD (20mm) to the MLE. The most resistant bacterial isolate to MBE was *K. pneumoniae* (11 mm) while the most sensitive was *M. luteus* (17mm). We suggested that MBE exhibited a bacteriostatic effect against *B. anthracis* because after 24 hours, the bacterium was observed to grow within the inhibition zones initially observed. *B. cereus* and *P. fluorescence* were sensitive to all the plant extracts while *P. aeruginosa* was resistant to all the plant extracts. Statistical analysis revealed that methanolic leaf extract of *L. camara* compared favourably with streptomycin in inhibiting the growth of *B. anthracis* (P= 0.09), MRSA (P=0.53), *B. substilis* (P=0.11) and *P. fluorescence* (P=0.09).

Table 1. The inhibition zone diameter showing Antibacterial activities of methanolic leaf extract, Aqueous leaf extract and methanolic berry extract of Lantana *camara Linn* against the bacterial isolates at 25 mg/ml

S/N	Bacteria isolates	Inhibition zone diameter (mm)**						
		MLE (25mg/ml)	*P*-value range	ALE (25mg/ml)	MBE (25mg/ml)	*P*-value range	Streptomycin (1mg/ml)	*P*-value range
	GRAM POSITIVE BACTERIA							
1.	*Corynebacterium pyogenes*(LIO)	$12 \pm 0.0^{a,h,k,p,\lambda,\varphi}$	.0005 - .01	-	-	-	$20 \pm 0.0^{b,j,m,r,t,w,\beta}$	.0005 - .04
2.	*Bacillus cereus* (NCIB 6349)	$17 \pm 0.6^{c,\dagger,n,u,x}$	.0005 - .04	11 ± 0.0	$12 \pm 0.0^{d,L,\Delta}$	.0005 - .001	$21 \pm 1.5^{e,g,o,t,\beta}$	.0005 - .04
3.	*Bacillus stearothermophilus*(NCIB 8222)	$18 \pm 0.0^{\dagger,c,n,s}$	.0005 - .049	-	-	-	$24 \pm 0.0^{g,e,o,t}$	.0005 - .02
4.	*Micrococcus luteus*(NCIB 196)	$12 \pm 0.0^{h,a,k,p,\lambda,\varphi}$	.0005 - .01	-	17 ± 0.0^{I}	.0005	$18 \pm 0.0^{j,b,m,r,w,\beta}$	.0005 - .003
5.	*Bacillus anthracis*(LIO)	$12 \pm 2.8^{k,a,h,p,\Omega,\varphi}$	.0005 - .02	-	$12^{*} \pm 0.0^{L,d,\Delta}$	.0005 - .001	$19 \pm 0.7^{m,b,j,r,w,\beta}$	.0005 - .02
6.	*Clostridium sporogenes*(NCIB 532)	$16 \pm 0.0^{n,c,\dagger,u,x,z,\varphi}$	.0005 - .01	11 ± 0.0	-	-	$24 \pm 0.0^{o,e,g,t}$	.0005 - .02
7.	*Bacillus polymyxa*(LIO)	$13 \pm 0.0^{p,a,h,k,z,\varphi}$	.0005 - .049	-	$15 \pm 0.0^{q,\varpi}$	.0005	$18 \pm 0.0^{r,b,j,m,w,\beta}$	.0005 - .003
8.	Methicillin resistant *Staphylococcus aureus*, MRSA (LIO)	$20 \pm 0.0^{s,\dagger}$	.0005 - .02	-	-	-	$22 \pm 4.5^{t,e,g,o,\beta}$	.0005 - .01
9.	*Bacillus substilis*(NCIB 3610)	$15 \pm 2.5^{u,c,n,x,z,\varphi}$	.0005 - .04	-	13 ± 0.0^{I}	.0005 - .001	$18 \pm 0.6^{w,b,j,m,r,\beta}$	.0005 - .01
	GRAM NEGATIVE BACTERIA							
10.	*Escherichia coli* (NCIB 86)	$16 \pm 0.7^{x,c,n,u,z,\varphi}$	.0005 - .049	-	-	-	11 ± 0.0^{y}	.0005
11.	*Klebsiella pneumoniae* (NCIB 418)	$15 \pm 0.0^{z,n,p,u,x,\varphi}$	.0005 - .04	-	$11 \pm 0.7^{\Delta,d,L}$	.0005	-	-
12.	*Proteus vulgaris* (LIO)	$10 \pm 2.8^{\Omega,a,h,k}$	.0005 - .02	-	-	-	$16 \pm 0.0^{\beta,j,m,r,w}$	.0005 - .003
13.	*Pseudomonas fluorescence*(NCIB 3756)	$14 \pm 2.8^{\varphi,a,h,k,n,p,u,x,z}$	.0005 - .01	10 ± 0.0	$15 \pm 0.7^{\varpi,q}$	.0005	$20 \pm 0.7^{\psi,b,e,j,m,r,t,w}$	.0005 - .02
14.	*Pseudomonas aeruginosa*(NCIB 950)	-	-	-	-		-	-

*MLE: Methanolic leaf extract; ALE: Aqueous leaf extract; MBE: Methanolic berry extract; LIO: Locally isolated organisms; NCIB: National collection of industrial bacteria; * Bacteriostatic; ** Mean of three replicates; - = No activity, P=0.05*

Comparison of mean IZDs of bacterial isolates when tested with MLE, MBE and Streptomycin: Isolates with different superscript are significantly different while ones with same superscript are statistically similar.

3.2 Phytochemical Analysis

The *L. camara* extracts were observed to have broad spectrum activities and therefore may be used in the treatment of infections caused by sensitive study bacteria.

The result of the phytochemical analysis, Table 2, reveals that flavonoids, saponins and alkaloids were present in all the plant extracts while tannin and terpenoid were present in only MLE and MBE respectively. However, phlobatanins, cardiac glycosides and steroids were absent in all the plant extracts. The presence of phytochemical compounds in *L. camara* has been attributed to most of its biological and antimicrobial activities [8]. Furthermore, antimicrobial activities of plant berries have been attributed to phenolic compounds such as flavonoids, phenolic acids, lignans, stilbenes and polymeric tannins present in them. The exhibited antimicrobial properties of *L. camara* can be attributed to the activities of saponins complemented with other polyphenols such as flavonoids and tannins present in the extracts. Tannins have been reported to possess antimicrobial properties and they exert their antimicrobial activities by inhibition of extracellular microbial enzymes, metabolism [7] and cell protein synthesis as well as possession of antioxidant properties [24]. Furthermore, saponins act by lysing bacterial cell membrane while flavonoids are synthesized by plants against microbial infections [16]. Although the mode of action of terpenoid is not well known, it is however believed to block cell division by inhibiting DNA synthesis in Gram positive bacteria and macromolecular synthesis in *B. substilis* [25].

Table 2. Phytochemical analysis of extracts of *Lantana camara* Linn

TEST	MBE	MLE	ALE
Phlobatannins	-	-	-
Flavonoids	+	+	+
Cardiac glycosides	-	-	-
Tannins	-	+	-
Terpenoids	+	-	-
Saponins	+	+	+
Steroids	-	-	-
Alkaloids	+	+	+

MLE: Methanolic leaf extract; ALE: Aqueous leaf extract; MBE: Methanolic berry extract, + = Present - = Absent

3.3 Mineral Content and Proximate Nutrient Analysis

In this study, the mineral content analysis as shown in Fig. 1 revealed that the powdered leaf and berry samples contain high amount of calcium (Ca), potassium (K) and phosphorus (P). Calcium has been described as essential element in diet usually required for proper growth, bone, teeth and muscle formation. The magnesium (Mg) and zinc (Zn) contents of the berries (869.2ppm and 784.9ppm respectively) is more than that of the leaves (750.1ppm and 518.7ppm respectively). Zinc is an important component of enzymes and it is also important in genetic expression including polynucleotide transcription and translation in humans. The leaves and berries also contain sodium (Na), an important mineral that is useful in lowering blood pressure. Iron (Fe), an important component of human haemoglobin, copper (Cu) which is important in the oxidation of unsaturated fatty acids [26] and manganese (Mn) were present in trace amount. The absence of lead (Pb) is of important benefit because it is a toxic heavy metal even at low concentration [27].The proximate analysis of the powdered leaf and berry samples as shown in Fig. 2 revealed that

each sample contains considerable amount of nutrients. The percentage content of carbohydrate in the samples was the highest while the amount of fat was relatively low. Carbohydrate and lipids are a good source of energy. The presence of fibre in the leaves (13.1%) and berries (12.8%) of *L. camara* suggests its importance in the absorption of trace elements in the guts and reduction of absorption of cholesterol [27]. The nutrient content of the leaf was observed to be more compared to the berry. This may be due to the more photosynthetic activity of the leaf compare to the berry that is just a fruiting structure of the plant.

The result of the mineral composition and proximate analysis of *L. camara* leaves reported in this study is similar to report of a study conducted by Ojo et al. [28].

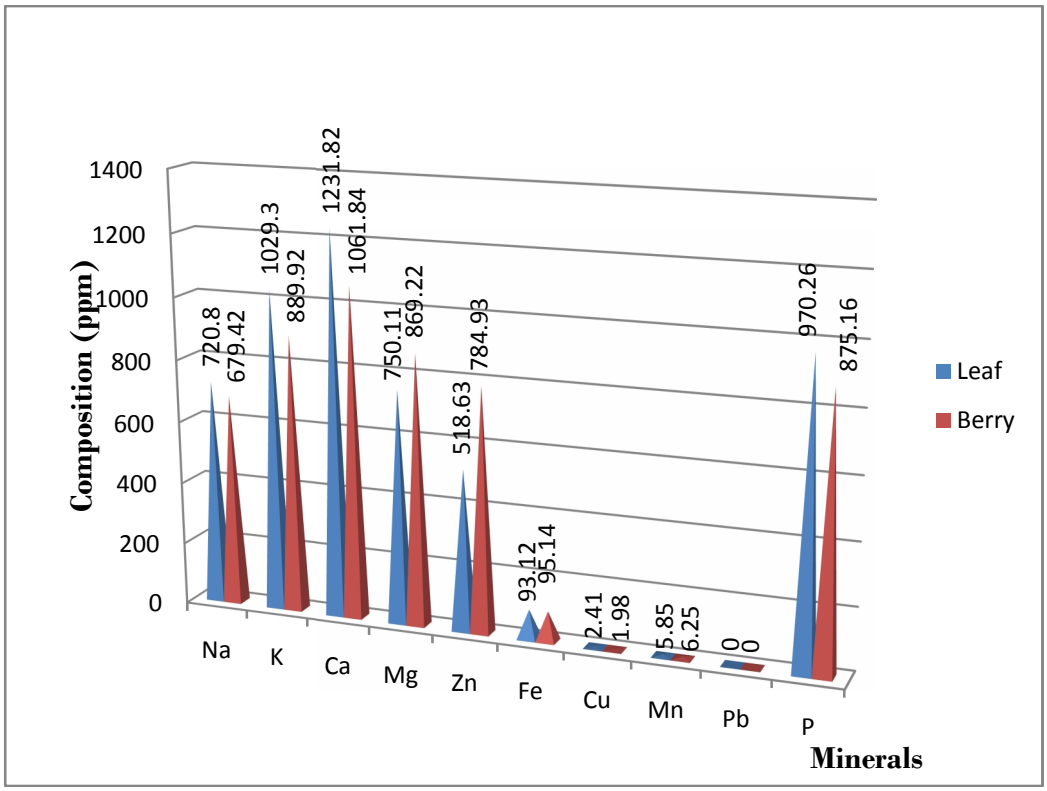

Fig. 1. Mineral content of *Lantana camara Linn* leaf and berry

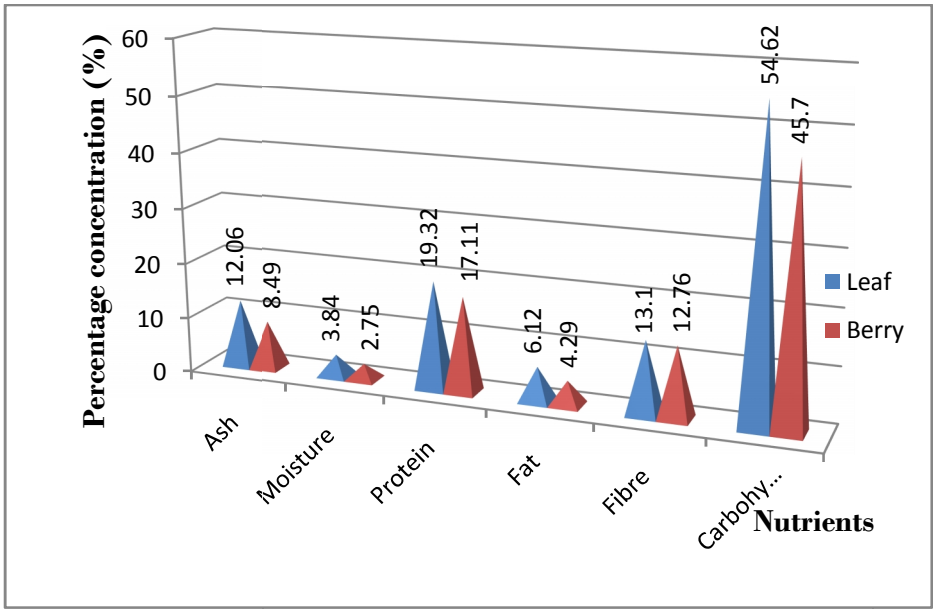

Fig. 2. Proximate nutrient analysis of *Lantana camara Linn* leaf and berry

4. CONCLUSION

This study showed that *L. camara Linn* contains phytochemical compounds with antibacterial activities against the sensitive study bacteria. Moreover, the methanolic leaf extract of *L. camara* is active against MRSA, pathogenic bacteria that is versatile in developing resistance to different classes of antibiotics as well as capable of causing severe nosocomial and community-acquired infections.

This study showed that the extracts of *L. camara* also possess broad spectrum antibacterial activities. The mineral and proximate analyses also revealed that *L. camara* leaf and berry are of important nutritional value. Pharmacology and toxicology of the *L. camara* should be further studied to determine how it can be utilized to treat bacterial infections.

CONSENT

Not Applicable.

ETHICAL APPROVAL

Not Applicable.

ACKNOWLEDGMENT

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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