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Bactericidal effect of the aqueous extract of the leaves of *Lantana camara* L. (Verbenaceae), a plant used in Benin in the treatment of skin infections.

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ABSTRACT

Objective: *Lantana camara* is a tropical plant whose leaves are used in Benin in traditional medicine to treat skin infections. This work aimed to test *in vitro* the effectiveness of the aqueous extract of *Lantana camara* leaves in the treatment of these infections.

Method and results: The aqueous extract of *Lantana camara* leaves were incubated for 24 hours at a dose of 50 mg/ml with strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two bacteria often isolated from skin infections. After incubation, the bacteria were cultured. The extract was bactericidal for all strains of *Staphylococcus aureus* and not for those of *Pseudomonas aeruginosa*. These strains were tested resistant to Amoxicillin + Clavulanic Acid. Then, the extract was administered for 28 days to Wistar rats at a dose of 300 mg/Kg/day. The blood leukocyte count of the rats was not significantly changed by the extract administered.

Conclusion and application of results: The aqueous extract from the leaves of *Lantana camara* was bactericidal against strains of *Staphylococcus aureus*. This result offered good prospects for the treatment of infections caused by Gram-positive cocci, a category to which *Staphylococcus aureus* belongs. The bactericidal effect was not noted on strains of *Pseudomonas aeruginosa*, which was part of the gram-negative bacilli. The extract administered *in vivo* to Wistar rats did not alter the leukocyte profile, suggesting its efficacy *in vivo* was due directly to its bactericidal

property and not to stimulation of the immune system. It could therefore be used not only in the treatment of internal infections, but also in that of skin infections by application to these lesions caused by these bacteria. Since the strains of *Staphylococcus aureus* used were resistant to Amoxicillin + Clavulanic Acid, the extract could be turned into an improved traditional medicine to offer an alternative to the problem of antibiotic resistance.
Keywords : *Lantana camara*, Staphylococcus aureus, *Pseudomonas aeruginosa*.

INTRODUCTION

Infectious diseases are caused by different types of pathogenic microorganisms, such as bacteria, viruses, parasites or fungi (WHO, 2015). These infectious diseases cause more than 17 million deaths per year worldwide and represent 43% of deaths in developing countries (WHO, 2015). They represent a major public health problem in the world and especially in developing countries where they are endemic (Khabbaz *et al.,* 2014). Bacterial infections are responsible for 70% of these deaths (Gangoue, 2003). The discovery of antibiotics significantly reduced the spread of these pathologies. The remarkable effectiveness of these antibiotics was accompanied by their massive and abusive use leading to the emergence of bacterial resistance (Ben *et al.*, 2005). This resistance contributed to making pathologies linked to microbes the leading cause of death in the world, killing more than 50,000 people per day (Ahmad *et al.,* 2001) and contributing to the need to search for new molecules. Medicinal plants remain potential sources of new active molecules. According to WHO (2002), an estimated 80% of the African population engaged in this practice for their own health. Among the many exploited species was *Lantana camara* L. This plant was used in the traditional treatment of diarrhoea, microbial infections, rashes and dermatoses. Moreover, recent studies revealed the hidden potential of this plant, which deserves investigation in the search for more effective antibacterial biomolecules (Z. Tonzibo *et al.*, 2006; Dèhou *et al.,* 2018). In order to broaden the spectrum of antibacterial action of *Lantana camara* and to identify the best method of concentration of active ingredients, this project was undertaken in order to study the antibacterial effect of aqueous extracts of the leaves of *Lantana camara* on two germs responsible for skin infections in humans: *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

MATERIAL AND METHODS

Plant material: The plant material consisted of the leaves of *Lantana camara*. The leaf sample was collected in the commune of Abomey-Calavi, in southern Benin. It was then identified and certified at the National Herbarium of Benin under the number YH 512 / HNB. After drying at laboratory temperature (20-25 ° C), they were ground into powder using a SONIK-SB brand mixer and stored in suitable jars in the laboratory.

Preparation of the aqueous extract: Fifty**(**50) g of powder from the *Lantana camara* leaves were boiled in 500 ml of distilled water in a 1000 ml flask for 30 minutes. After cooling, the mixture is filtered through a Bushner. This operation was repeated six times for a total mass of 300 g (Koudoro et al, 2014; Tchogou et al, 2016).

-Microbial strains: Strains *of Pseudomonas aeruginosa* (Gram-negative bacillus) and *Staphylococcus aureus* (Gram-positive cocci) isolated from skin infections and abscesses in the clinical biology laboratory of the Saint Jean de Dieu hospital in Boko, Benin, were used for antimicrobial testing. They were stored in nutrient agar-based media at 4 ° C prior to use.

Culture media and antibiotics: Mueller-Hinton (MH) broth was used for the culture of

bacteria. Eosin Methylene Blue (EMB) culture medium was used to isolate *Pseudomonas aeruginosa* and Chapman's medium for Staphylococcus aureus. The culture media and antibiotic discs namely Norfloxacin and Amoxicillin + Clavulanic Acid used as control came from the companies Biomerieux® and Biorad®.

Animal material: The animal material consisted of albino Wistar rats with an average body weight of 130 to 157 g, having free access to water and food and acclimatized to the conditions of breeding of the animal house of the laboratory of Molecular Pathophysiology and Toxicology of the Faculty of Sciences and Techniques (FAST) of the University of Abomey-Calavi UAC) in the Republic of Benin. The breeding took place in a well ventilated room, with a day-night rhythm of 12 hours. The animals were kept in wire mesh cages with feeders and drinkers. Their daily diet consisted of a mixture of foods in the form of croquettes and marketed by Veto Services (Benin). The enclosure was regularly cleaned to ensure optimal development of the animals and avoid infections.

Anti bacterial efficacy test: A colony of *Staphylococcus aureus* or *Pseudomonas aeruginosa* was inoculated into a tube containing 9 ml of MH broth. Two successive dilutions of 10 µl of this suspension in 9 ml of MH broth were performed. 200 µl of the dilution were incubated with 200 µl of plant extract at 100 mg / ml at 37 ° C for 18 hours. After incubation, two successive dilutions were then carried out: 10 µl of the suspension in 8990 µl of physiological water, then 40 µl in 8960 µl of sterile physiological water. After this second dilution, the suspension was inoculated for over 18 h on EMB medium for *Pseudomonas aeruginosa* or on Chapman medium for Staphylococcus aureus. Colonies were then counted by comparing to controls. For controls, the extract was replaced with sterile distilled water or antibiotic discs (adapted from Anago et al, 2011).

Immune system stimulation test: The extract was administered daily for 28 days to Wistar rats at a dose of 300 mg / Kg of body weight. The white blood cell count were determined at the beginning and end of the experiment.

Statistical analysis: Means were compared using the Mann-Whitney test. The significance threshold was set at 5%.

RESULTS AND DISCUSSION

Results: Bactericidal activity of *Lantana camara*extract: Tables 2 and 2 showed the effects of *Lantana camara*extract on strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively.

Table 2: Result of *Lantana camara*extract on Staphylococcus aureus

Staphylococcus aureus		**Strain 1**	**Strain 2**	**Strain 3**
*Lantana camara*extract	Number of colonies counted	0	0	0
	Bactericidal effect	100%	100%	100%
Sterile distilled water	Number of colonies counted	> 100	> 100	> 100
	Bactericidal effect	0%	0%	0%
Norfloxacin	Number of colonies counted	0	0	0
	Bactericidal effect	100%	100%	100%
Amoxicillin + Clavulanic Acid	Number of colonies counted	> 100	> 100	> 100
	Bactericidal effect	0%	0%	0%

The extract was bactericidal on all strains of *Staphylococcus aureus* compared to the control, sterile distilled water which did not inactivate any of the strains of Staphylococcus aureus. These effects were compared with two antibiotics Norfloxacin and Amoxicillin + Clavulanic Acid. Norfloxacin inactivated all strains of Staphylococcus aureus, but Amoxicillin + Clavulanic Acid did not inactivate any of the strains of Staphylococcus aureus, which allowed to conclude that the strains of *Staphylococcus aureus* were resistant to Amoxicillin + Clavulanic Acid.

Table 2: Result of *Lantana camara*extract on *Pseudomonas aeruginosa*

Pseudomonas aeruginosa		Strain 1	Strain 2	Strain 3
Lantana camara extract	Number of colonies counted	> 100	> 100	> 100
	Bactericidal effect	0%	0%	0%
Sterile distilled water	Number of colonies counted	> 100	> 100	> 100
	Bactericidal effect	0%	0%	0%
Norfloxacin	Number of colonies counted	0	0	0
	Bactericidal effect	100%	100%	100%
Amoxicillin + Clavulanic Acid	Number of colonies counted	> 100	> 100	> 100
	Bactericidal effect	0%	0%	0%

The extract was not bactericidal on any of the strains of *Pseudomonas aeruginosa* as was the control, sterile distilled water. These effects were compared with the two antibiotics Norfloxacin and Amoxicillin + Clavulanic Acid. Norfloxacin inactivated all strains of *Pseudomonas aeruginosa*; however, Amoxicillin + Clavulanic Acid did not inhibit any strain of *Pseudomonas aeruginosa*.

Effect of *Lantana camara* aqueous extract on the immune system: The result of chronic administration of the extract to the immune system of rats was shown in Table 3.

Table 3: Number of blood leukocytes before and after treatment of rats with aqueous extract of *Lantana camara*

Immune cells	**Mean at day 0**	**Mean at day 28**	**P value**	**Difference**
Total number of blood leukocytes (G / L)	11.10 ± 2.40	9.0 ± 2.34	0.4	Not significant
Polynuclear neutrophils (G / L)	0.61± 0.17	0.97 ± 0.20	0.1	Not significant
Lymphocytes (G / L)	8.83 ± 0.73	6.38± 1.89	0.2	Not significant
Monocytes (G / L)	1.29 ± 0.15	0.65 ± 0.10	0.1	Not significant

At the start of the experiment on day 0, the mean number of leukocytes in the blood was 11.10 ± 2.40 G / L. The mean numbers of neutrophils, lymphocytes and monocytes were

0.61 ± 0.17 G / L, 8.83 ± 0.73 G / L and 1.29 ± 0.15 G / L, respectively. On day 28, these different values did not change significantly. This showed that the administration of the aqueous extract of *Lantana camara*did not influence the production of immune cells.

DISCUSSION

After culture, the bactericidal effect of the extract was complete on strains of *Staphylococcus aureus*. This study results agree with those obtained by Bangou (2012) who, after a study in Burkina Faso, indicated that *Lantana camara* could be used in the treatment of oral and skin diseases, dysentery, food poisoning and pneumopathies. On the other hand, on the strains of *Pseudomonas aeruginosa*, the number of colonies was greater than 100 per culture, thus showing that the strains of this bacterium were insensitive to the aqueous extract of *Lantana camara.* These results revealed that the aqueous extract of *Lantana camara* was sensitive to the gram-positive bacteria *Staphylococcus aureus* and possibly to other Gram-positive bacteria in general. The extract was insensitive to the Gram-negative bacteria *Pseudomonas aeruginosa*. These results agreed with those obtained by Nacoulma (1996) who, after a study on the same plant, indicated that the antimicrobial activity demonstrated would confirm the therapeutic use of Lantana camara leaves in traditional medicine in the treatment of oral diseases, coughs, lung disease, diarrhoea, influenza as an antibiotic. Our study showed that the effect would be directed against Gram-positive bacteria. In addition, the total number of blood leukocytes during chronic administration of the aqueous extract of the leaves of Lantana camara in vivo to Wistar rats did not change significantly. It was the same for those of polymorphonuclear neutrophils, lymphocytes and monocytes suggesting that the effect of the extract was not mediated by stimulation of immune cells. Because stimulation of immune cells during infection resulted in a significant increase in their number in the blood (Yang, 2014; and Sènou, 2017b). Therefore, we could conclude that Lantana camara leaves aqueous extract exhibited a direct bactericidal effect on *Staphylococcus aureus* and this effect was not mediated by the immune system stimulation. The extract of this plant could therefore be used to treat skin infections, in particular that caused by Staphylococcus aureus. These results complemented those of Kumarasamyraja et al. (2012), who reported antibacterial activity from some extracts of the leaves, flowers, roots and fruits of Lantana camara.

Moreover, we compared the effects of the extract to two commonly used antibiotics, Norfloxacin and Amoxicillin + Clavulanic Acid. Norfloxacin inactivated all strains of *Staphylococcus aureus* unlike Amoxicillin + Clavulanic Acid that did not inhibit any strain of Staphylococcus aureus. This revealed the resistance of these strains of *Staphylococcus aureus* to Amoxicillin + Clavulanic acid, which was however considered a potent antibiotic against Gram + cocci. These results showed that the use of the aqueous extract of Lantana camara leaves could offer an alternative to the problem of antibiotic resistance (Davies, 2010; Berendonk, 2015), a public health problem.

CONCLUSION AND APPLICATION OF RESULTS

This study contributed to a better understanding of the antibacterial activity of *Lantana camara*, a plant belonging to the Verbenaceae family. The study also promoted the Beninese pharmacopoeia as tools for the management of health problems in developing countries and the search for improved traditional medicines. In fact, the aqueous

extract of the leaves of *Lantana camara* was bactericidal on Staphylococcus aureus. The result thus supported the use of the plant in traditional medicine and required more in-depth research aimed at determining the compounds responsible for the observed activity. The use of aqueous extracts from *Lantana camara* leaves may offer an alternative to the growing problem of antibiotic resistance.

REFERENCES

Gangoue PJ. 2007. Caractérisation des bêtalactamases et leur inhibition par les extraits de plantes médicinales. Thèse de Doctorat en biochimie. Liège : Université de Liège-Belgique ; 104p.

Ben F, Rodan CH, Bougerra O, Sahnoun CH, Loussaies V, KacemM, Mastouri R, Tavka-Stanbouli M, Chakroun V, Bouzouaùîa N. 2005. Les bactéries multirésistantes isoléés chez les malades hospitalisés dans un service de maladies infectieuses. Tin Infectiol, 1(4) : 12-15.

Ahmed Abid, M. (1996). A taxonomic review of Lantana camara L. and monte videnSis (spreng.) briq. (verbenaceae) in australia. 17, 1-27P.

Nea fatimata (2021). Etude phytochimique et biologique de deux plantes médicinales de côte d'ivoire : lantana camara et lantana rhodesiensis (verbenaceae). Thèse de doctorat, université de liège – gembloux, université félix houphouët boigny – abidjan cocody.

Who. (2007). Who guidelines for assessing quality of herbal medicines with reference to contaminants and residues. World health organization, geneva, 105 p.

OMS, Stratégie de l'OMS pour la médecine traditionnelle pour 2002- 2005, Génère, (2002) 65 p.

Mindiédiba Jean Bangou, Martin Kiendrebeogo, Moussa Compaoré, Ahmed Yacouba Coulibaly, Nâg-Tiero Roland Meda, Norma Almaraz Abarca, Boukaré Zeba, Jeanne Millogo-Rasolodimbv and Odile Germaine Ncoulma (2011). Enzyme Inhibition Effect and Polyphenolic Content of Medicinal Plant Extracts from Burkina Faso. Journal of Biological Sciences 11 (1) : 31-38.

Kumarasamyraja, D., Jeganathan, N S., Manavalan, R. (2012). Pharmacological review of Lantana Camara .L. 01, 1-5P.

Nacoulma, O.G., 1996. Medicinal plants and their traditional uses in Burkina Faso. Ph.D. Thesis, University of Ouagadougou, pp: 328

Sènou, m., a.p. tchogou, c.p. agbangnan-dossa, t.v. dougnon, p. Ogué, a. Agossadou, a. Lalèyè, f. Loko, A. Agbonon, a. Sèzan, (2017a). Efficacy of ethyl acetate fraction of cocosnucifera's aqueous extract on the Treatment of anemia. International journal of pharmaceutical science invention, 9(9), 44-51.

Dubey, M., Sharma, S., Bhadauria, S. and Gautam, R.K. (2011). In vitro antibacterial activity of Lantana camara leaves hydrosol. Journal of pharmacy and research, 4(11) 3972-3974P.

David, M., Musyimi, G., Chesire, J., Sikuku, A and Daniel, K. (2002). Antimicrobial potential and screening of phytochemical compounds of Lantana camara Linn. Journal of Biological Science, Vol (3), 18-35P.

Erlânio, O., Sousa, O and José, G.M. (2012). Genus Lantana: chemical aspects and Biological activities. Brazilian Journal of Pharmacognosy, 22(5), 1155-1180P.

DOUGNON, G. & ITO, M. 2019. Sedative effects of the essential oil from the leaves of Lantana camara occurring in the Republic of Benin via inhalation in

mice. Journal of Natural Medicines 74, 159–169. DOI 10.1007/s11418-019-01358-9

René Dèhou, victorien Dougnon, Pascal Atchade, Cyrille Attakpa, Boris Legba, Lamine Baba-moussa et Fernand Gbaguidi, 2018. Étude phytochimique et toxicologique de cassia italica, momordica balsamina et ocimum gratissimum, trois plantes utilisées contre la gale au sud-bénin rev. Ivoir. Sci. Technol., 32pp 286 – 297.

Yang J, Zhang L, Yu C, Yang XF & Wang H. Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. Biomark Res 2014; 2: 1–9.

E. Anago, L. Lagnika, J. Gbenou, F. Loko, M. Moudachirou and A. Sanni (2011). Antibacterial Activity and Phytochemical Study of Six Medicinal Plants used in Benin. Pakistan Journal of Biological Sciences 14 (7) : 449-455.

Sènou M, Agbangnan DCP, Zime N'‘Gobi AR, Abissi GY, Agbogba F, Sèzan A, Loko F (2017b). In malaria, acute inflammation level depends on parasitaemia. International Journal of Pharmacy and Biological Sciences 7 (2): 23-30. P-ISSN: 2321-3272, E-ISSN: 2230-7605.

Davies J. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010;74: 417–433. 10.1128/MMBR.00016-10.

Berendonk TU, Manaia CM, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, et al. Tackling antibiotic resistance: the environmental framework. Nat Rev Microbiol. 2015;13: 310–317. 10.1038/nrmicro3439.

Koudoro, YA, Dedomè LSO, Yovo M, Agbangnan-Dossa CP, Tchobo PF, Alitonou GA, Avlessi F, Sohounhloué DCK, 2014. Chemical characterization, antiradical and antibacterial activities of extracts of the root bark of Cochlospermum planchoni of Benin. International Journal of Innovation and Applied Studies 7(4) : 1582-1594.

Tchogou, a.p., m. Sènou, t.v. dougnon, a. Agossadou, f. Assogba, e.g. kinsiclounon, e. Ewedjè, c.p. Agbangnan-dossa, j. Gbénou, a. Lalèyè, f. Loko, (2016). The aqueous extract of cocos nucifera l. (arecaceae) effectively treat induced anemia. Experimental study on wistar rats. International journal of Biology, 8(3), 5-54.