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# **Research Article**

# IN-VIVO AND IN-VITRO ANTIMICROBIAL EFFECT OF THE AQUEOUS LEAF EXTRACT OF CITRILLUS COLOCYNTHIS LINN-SCHRAD (CUCURBITACEAE)

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#### Abstract

The rampant emergence of multi-drug and cross-resistant strains of pathogenic-microbes is rapidly limiting the effectiveness of anti-microbial agents. Plants with antimicrobial remedies are increasingly locally being used alone or as adjuncts. In-vivo and in-vitro antimicrobial activity of the aqueous leaf-extract of Citrillus-colocynthis were thus, investigated.For in-vitro agar-well diffusion assay, 6-clinical-isolates and 2-standards were used whereby the mean diameter of growth-inhibition zones (n = 3) were compared against controls. For in-vivo assay, the mid-dorsal skin of 30 rats wounded by shaving and infected by subcutaneous injection of Staphylococcus-aureus was used. The rats in 6-groups of 5 were then given immediate post-infection subcutaneous-treatment at the infected-sites with distilled-water (10ml/kg), extract-doses, 2mg/ml ciprofloxacin and 500mg/kg extract plus 2mg/ml ciprofloxacin respectively and checked 48hours later for visible inflammatory-signs. The infected skin-sites from 3 rats in every group were removed and homogenized in 2ml sterile-water from which a 1:10 diluted-sample for each-group was made, incubated for 24hours at 37°C, the numbers of viable-bacteria were counted. Ciprofloxacin inhibited growth of all other organisms (standards and isolates) except Escherichia-coli isolate, but, which was significantly(P 0.001) susceptible only to the 100% extract-dose. The extract at 25%, 50% and 100% significantly and dose-dependently inhibited growth of one standard (Staphylococcus-aureus), two gram-positive and two gram-negative isolates.For invivo studies, the negative-control group had 6600±871.8 bacteria counts, while ciprofloxacin and the extract-doses had significantly highly-reduced dose-dependent bacteria counts with extract plus ciprofloxacin having the lowest count. Citrillus-colocynthis aqueous leaf-extract demonstrated a dose-dependent anti-microbial activity in both invitro and in-vivo models. The extract augmented the effect of ciprofloxacin in a synergistic manner.

Keywords: Antimicrobial activity, Citrullus colocynthis, Growth inhibition, Skin infection, Staphylococcus-aureus.

#### **INTRODUCTION**

The use of plants as source of remedies for the treatments of many diseases is as old as life itself and people of all continents have this old tradition and had successfully used natural native plants or their extracts for many infections and diseases.<sup>[1]</sup> The current research interest in medicinal plants is not only on their presumed safety, but also because of the presence of various complex chemical metabolites of diverse medical importance contained in these plants such as alkaloids, glycosides, flavonoids, saponnins, tannins, carbohydrates etc.<sup>[2,3]</sup> Plants based natural constituents provide the basic raw materials for indigenous pharmaceutical, perfumery, flavor

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and cosmetics industries; and as more discoveries of both the enormous therapeutic potentials of plant herbs and serious side effects of many of the synthetic drugs in use emerge, the need to screen various plants for their medicinal values becomes imperative. For instance, the rampant emergence of multi-drug and cross-resistant strains of pathogenic-microbes is rapidly limiting the effectiveness of many anti-microbial agents and plants with antimicrobial remedies are increasingly locally being used alone or as adjuncts in the treatment of microbial infections.

A number of plants from different families of Angiosperms have been reported to show antimicrobial activity.<sup>[4]</sup>

*Citrillus colocynthi*is is an important medicinal plant belonging to the family of Cucurbitaceae. It is annual or perennial (in wild), herbaceous, stems are angular and rough, leaves rough, flowers monoecious, fruit is bitter flavored, nearly globular. Native to dry areas of North Africa, being common throughout the Sahara, areas of Morocco, Egypt and Sudan, eastward through Iran to India, Pakistan, Afghanistan and other parts of tropical Asia.<sup>[5]</sup> It seeds are rich in oil and protein. It grows wild in sandy shore under xerophitic conditions.<sup>[6]</sup>

It is a well recognized plant in the traditional medicine and was used by people in rural areas as a purgative, anti-diabetic, rheumatism, snakebite, anti-tumour (especially of the abdomen) and insecticides. The fruit had been used medicinally since ancient times for the treatment of microbial diseases, ulcer, inflammation, jaundice and urinary diseases in Asian and African countries.<sup>[7]</sup>

Recent studies have shown that *C. colocynthis* can have an anti- diabetic, carcinogenic, antioxidant, anti-bacteria effects.<sup>[8-11]</sup>.

In the laboratory, leaves of *C. colocynthis* had anti-inflammatory,<sup>[12]</sup> antidiabetic,<sup>[13]</sup> anti oxidant<sup>14</sup> and local anaesthetic activities.<sup>[14]</sup>

Thus, there is an ever-increasing demand for more and more drugs from plant sources<sup>[15]</sup> and as Citrillus-colocynthis is commonly used in Asian and African countries to treat infections, the extent of this effect was investigated.

#### MATERIALS AND METHODS

#### Materials

Macconkey agar, (4%) saboraud dextrose agar, mueller hinton agar and broth (Oxoid Laboratories, England); ciprofloxacin infusion (Fresenius Kabi Pvt Limited, Germany), 70% ethanol (New Health Way Co. Limited, Nigeria).

#### **Plant Material**

The fresh leaves of actively growing *Citrillus colocynthis* were collected from Ikire Township, Osun state, Nigeria by Mr. T. K. Odewo. The plant was first identified at the field using standard keys and descriptions.<sup>[16-18]</sup> Its botanical identity was further confirmed and authenticated at the Herbarium section of the department of Botany, University of Lagos, Akoka, Lagos, Nigeria. **Volume 4, Issue 1, 2015** 



Voucher specimens (LUH 3582) were preserved and stored at the herbarium for future references.

#### **Extraction of plant material**

116g of crushed leaves was weighed and soaked in 1.5 litres of distilled water in a conical flask and boiled for 30 minutes at  $60^{\circ}$ C on a hot plate. The boiled leaves were macerated for 48 hours and was then filtered using clean white piece of cloth. The filtrate was collected into clean stainless plates and dried in the oven at temperature of  $40^{\circ}$ C. This yielded a brown residue (24.18%) which was kept in a sterile bottle in the refrigerator until ready for use.

#### **Bacterial strains**

Six clinical-isolates (*Staphylococcus aureus. Klebsiella pneumonia. Escherichia coli. Salmonella typhi. Pseudomonas aeruginosa, Bacillus subtilis*) and 2-standards of the America Type Culture Collection (ATCC) were used in this study. The test organisms were obtained from the Research Laboratory of Medical Microbiology & Parasitology of the College of Medicine, University of Lagos, Idi-araba, Lagos.

#### METHODS

# *IN-VITRO* ANTIMICROBIAL EFFECTS OF AQUEOUS LEAF EXTRACT OF C. COLOCYNTHIS USING AGAR-WELL DIFFUSION ANTIMICROBIAL ASSAY

For in-vitro agar-well diffusion assay, the anti-microbial effect of *C. colocynthis* leaves extract was tested for using method described by.<sup>[19]</sup>

Six clinical-isolates and two standards were used whereby the mean diameter in millimetre of growth-inhibition zones (n = 3 experiments per concentrations of 12.5%, 25%, 50% and 100%) were compared against controls (distilled-water and ciprofloxacin). A volume of 0.1 ml of the different extract concentrations 100%, 50%, 25%, 12.5% were put into 4 holes in each test organisms' culture media respectively against sterile water and ciprofloxacin in the 5<sup>th</sup> and 6<sup>th</sup> holes. Three different plates of similar experiment was made for each of the organisms and kept for 1 hour in the refrigerator at 6<sup>o</sup>C to diffuse prior to overnight incubation at 37<sup>o</sup>C for 18-24 hours. The diameter of the growth inhibition zones were measured and recorded from the 3 experiments of each concentration in the three plates and the mean calculated for each drug concentration.

# *IN-VIVO* ANTIMICROBIAL EFFECTS OF AQUEOUS LEAF EXTRACT OF C. COLOCYNTHIS USING RAT SKIN WOUND INFECTION

The mid-dorsal skin of 30 rats were shaved and infected by subcutaneous injection of  $1 \times 10^{10}$  colony forming units per rat (cfu / rat) of  $100\mu$ l of *Staphylococcus aureus*. The rats were then divided into 6 groups of 5 rats per group for immediate post infection subcutaneous treatment of the infected sites with 10 ml/kg distilled water (negative control), extract doses (100, 250 and **Volume 4, Issue 1, 2015** 

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500 mg/kg), 2 mg/ml ciprofloxacin (positive control) and 500 mg/kg extract plus 2 mg/ml ciprofloxacin respectively. Development of inflammatory reactions such as swelling, redness, skin lesions and / or abscesses within 48 hours at the infected sites was noted for the various groups. The bacteria numbers present at the infected sites were then quantified by which three rats from each group were sacrificed and disinfected with 70% ethanol prior to removal of the infected skin sites with underlying tissues of each of the 3 rats of a group and homogenizing in 2 mls sterile water. The number of S. *aureus* colony forming units (cfu/g) per gram of tissue was then obtained from the 1:10 diluted sample of each of the groups on Mac-Conkey agar 24 hours /  $37^{0}$ C incubated plates.<sup>[20]</sup>

#### **RESULTS AND DISCUSSION**

Table 1 summarizes the microbial growth inhibition by aqueous extract of *C. colocynthis*. The extract at 25%, 50% and 100% significantly and dose-dependently inhibited growth of one standard (*Staphylococcus-aureus*), the two gram-positive and two gram-negative isolates (*Klebsiella-pneumonia, Pseudomonas-aeruginosa*). The 12.5% extract-dose inhibited only the *Staphylococcus-aureus* isolate significantly (P 0.001). The extract did not inhibit growth of standard *Escherichia-coli* and *Salmonella-typhi* isolates both of which are gram negative bacteria. This is because gram negative bacteria have an outer membrane, which make their cell wall impenetrable to anti-microbial agents, it is possible that the apparent ineffectiveness of *C. colocynthis* was largely due to this permeability barrier. Gram positive bacteria are only composed of peptidoglycan cell wall and so are more susceptible to anti-microbial agents.<sup>21</sup> A higher concentration of the extract is required to inhibit the growth of *Salmonella typhi* and *ATCC E.coli*. Ciprofloxacin inhibited growth of all other organisms (standards and isolates) except *Escherichia-coli* isolate, but, which was significantly (P 0.001) susceptible only to the 100% extract-dose.

For *in-vivo* studies, the negative-control group had  $6600\pm871.8$  bacteria counts, while the extract-doses had significantly highly-reduced dose-dependent counts of  $3333\pm290.6$ ;  $2800\pm152.8$ ;  $2200\pm57.74$  respectively; with ciprofloxacin having  $1667\pm145.3$  and extract plus ciprofloxacin,  $933.3 \pm 145.3$ .

The anti-microbial investigation of the aqueous extract revealed that the extract was active against *Staphyloccocus aureus* inoculated rats at higher concentration and when used in combination with ciprofloxacin.

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Table 1: Growth Inhibitory Effect of the Aqueous Leaf Extract of C. Colocynthis on the

**Bacterial Test Organisms** 

ORGANISMS	$Mean \pm SEM \text{ of Growth Inhibitions (mm) of } Drug \text{ CONCENTRATIONS (mg/ml) at :}$					
	500 Ext.	250 Ext.	125 Ext.	61.25 Ext.	2.0 Cipro.	Distl H <sub>2</sub> O
	(100%)	(50%)	(25%)	(12.5%)		
Staphylococcus aureus	13±0.58***	11.3±0.88***	9.6±0.81***	6.5±1.39***	21.3±0.88	_
	(61.03%)	(53.05%)	(45.07%)	(30.51%)	(100%)	
Escherichia coli	11.4±0.62	_	_	_	_	_
Salmonella typhi	-	_	-	_	25.7±1.15	_
Klebsiella	10.6±1.20***	8.6±1.20***	11±1.53***	_	35±1.16	_
pneumonia	(30.29%)	(24.57%)	(31.43%)			
Pseudomonas aeruginosa	10±1.16***	8±1.15***	7.6±0.95***	_	34±1.16	_
	(29.41%)	(23.53%)	(22.35%)			
Bacillus subtilis	11±1.56***	9.3±0.95***	5.6±0.87***	_	25.3±0.44	_
	(43.48%)	(36.76%)	22.13%)			
ATCC Escherichia coli 25922	-	-	-	-	21.6±0.83	-
ATCC Staphylococcus aureus 25923	12.6±0.70***	10±1.16***	8±1.15***	_	21±1.16	_
	(55.24%)	(47.62%)	(38.09%)			

(Dunnett post hoc: P<0.001 wrp to positive standard)

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Treatment Drug Concentrations in mg/ml



#### CONCLUSION:

Citrillus colocynthis aqueous leaf extract demonstrated a dose dependent antimicrobial activity in both in-vitro and in-vivo models and the doses used showed more effect on gram-positive organisms suggesting that higher concentrations may be required for the more resilient gramnegatives organisms. The extract augmented the effect of ciprofloxacin in a synergistic manner.

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