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ANTIBACTERIAL EFFECT OF CAPSICUM ANNUM ON *PSEUDOMONAS AERUGINOSA* AND *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Studies on extract of *Capsicum annum* (red or sweet pepper) were carried out to assess its inhibitory effect on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This was done by preparing crude extract of the pepper with sterile distilled water. The microbes were treated with the extract by agar diffusion and tube dilution methods. The crude extract showed considerable inhibitory effect on the bacteria with 16 mm and 24 mm diameters in the zones of inhibition for *P. aeruginosa* and *S. aureus* at 24 hr of incubation respectively. At 48 hr of incubation, the diameter in the area of growth inhibition for *S. aureus* decreased to 11 mm and that of *P. aeruginosa* increased to 21 mm. Minimum inhibitory concentrations of the pepper were 335 mg/ml and 402 mg/ml for *P. aeruginosa* and *S. aureus* respectively.

INTRODUCTION

Capsicum annum (red or sweet pepper), a red pod-like berry fruit, like other *Capsicum* species (chillies) has many uses all over the world. The pepper is nutritious. A 100 g of it contains protein (2%), fat (0.8%), carbohydrate (10%), fibre (2.6%), calcium (29 mg), phosphorus (61 mg), iron (2.6 mg), ß-carotene equi. (180 µg), thiamine (0.12 mg), riboflavin (0.15 mg), niacin (2.2 mg), ascorbic acid (140 mg), water (86 ml) and calories (48 joules)[1]. It is mainly used in raw, ripe and dried forms for flavouring stew, curries, chutney and vegetable dishes or salads. Its cooked leaves are eaten raw. Fine powder of the chilli is sold as Cayenne pepper and the extracts have many pharmaceutical uses. The red pepper is a pungent stimulant, stomachic and carminative. In small doses it helps in the secretion of saliva and gastric juice, and also induces peristaltic movements[2,3]. Lowenfeld and Lowenfeld[4] reported that *C. annum* is used for the treatment of diarrhoea, dropsy, toothache, relaxed throat, gout and as liniment.

Little or no information is available on medicinal use of the plant in Nigeria. In a preliminary research study carried out on antibacterial activities of some plants used as condiments and spices in Nigeria, it was shown that the *C. annum* highly inhibited the growth of *Pseudomonas aeruginosa* and *Escherichia coli*[5]. In this study antagonistic effect of the sweet pepper's extract was examined on *Staphylococcus aureus* and *P. aeruginosa*. In an attempt to quantify the effective dose of the red pepper against Gram positive and Gram negative human pathogenic bacteria, minimum inhibitory concentrations of the *C. annum* for *P. aeruginosa* and *S. aureus* were determined.

MATERIALS AND METHODS

Sources of Materials

Fresh *Capsicum annum* (Sweet pepper) was obtained from the "Oba" market in Akure, Ondo State, Nigeria. The human pathogenic bacterial strains: *Pseudomonas aeruginosa* and *Staphylococcus aureus* were provided by University College Hospital, Ibadan, Oyo State, Nigeria.

Preparation of Extract

A blender was surface-sterilized by pouring absolute ethanol into it. It was left for 30 min., drained and allowed to dry. Raw extract was made by grinding 100 g of the sweet pepper in 150 ml sterile distilled water. Crude extract was prepared by filtering the raw extract through a sterile sieve with pore size of 0.1 mm.

Agar Diffusion Test

An aliquot (0.5 ml) of 18 hr old nutrient broth culture (OD_{600}, 0.45) of each of the bacteria was spread on nutrient agar. An hole was bored into the agar with 14 mm cork borer and filled with the crude extract. Control plate contained sterile distilled water instead of the extract. Another control was set up as the test but lacked the bacteria. All plates were incubated at 37°C for 24 to 48 hr and

observed for clear zone which indicates sensitivity of the test organisms to the pepper. Degree of sensitivity was determined by measuring the diameter in the zone of inhibition in millimetre. This procedure was carried out three times.

Determination of Minimum Inhibitory Concentration

This test was carried out by tube dilution method with crude sweet pepper extract using *P. aeruginosa* and *S. aureus*. A 0.5 ml of 18 hr *P. aeruginosa* (20 x 10^7 cells) and *S. aureus* (26 x 10^7 cells) were added to peptone water contained in a test tube. Crude extract was added to obtain varying final concentrations of 0.067, 0.134, 0.201, 0.268, 0.335, 0.402, 0.469 and 0.536. Two sets of control tubes were made up as the test tubes. A set of the control tubes contained nutrient broth substituted for the bacterium and the other set lacked the extract replaced with sterile nutrient broth. All tubes were incubated at 37°C for 24 hr. Transparency at the top of the liquid in the tube was recorded as inhibition. Aliquot (0.1 ml) of this liquid was inoculated into nutrient agar in order to confirm death of the bacterial cells. Concentration of the sweet pepper extract in tube corresponding to where complete death of the organism started was noted as the point of minimum inhibitory concentration.

The above described procedure used for minimum inhibitory concentration (MIC) test was repeated to determine the specific MIC for each of the bacteria with final extract concentrations of 0.268, 0.282, 0.294, 0.308, 0.321 and 0.335 g/ml for *P. aeruginosa* and 0.335, 0.348, 0.363, 0.377, 0.391 and 0.402 g/ml for *S. aureus*.

RESULTS AND DISCUSSION

S. aureus was more sensitive than *P. aeruginosa* to the crude sweet pepper extract. Zone diameter of growth inhibition of the extract was 24 mm for *S. aureus* and 16 mm for *P. aeruginosa* at 24 hr of incubation. At 48 hr of incubation, zone diameter of growth inhibition for the *P. aeruginosa* increased to 21 mm and that of *S. aureus* decreased to 11 mm. This suggests that the *C. annum* is bacteriostatic on *S. aureus* and bacteriocidal or bacteriolytic in action against *P. aeruginosa*. The antibacterial activity of the pepper is due to the presence of capsine in its flesh, seeds and placenta which also accounts for its strong taste[6].

In the tube dilution MIC test, *P. aeruginosa* and *S. aureus* appeared susceptible to the crude sweet pepper extract at the minimum inhibitory concentrations of 335 mg/ml and 402 mg/ml for the former and latter bacteria respectively.

The higher concentration of the extract for its antagonistic activity against *S. aureus* than that of the second bacterium could be because the pepper was bacteriostatic on it. However, these concentrations are higher than the standard value (30 mg/ml) recommended for MIC and those of many antibiotics such as gentamycin, tetracycline, erythromycin, ampicillin, tobramycin, kanamycin and chloramphenicol commonly used against these two organisms[7]. This could be attributed to the reason that the sweet pepper is a plant containing other consitituents while the conventional antibiotics are purified substances synthesized by microorganisms wholly or partially from chemical compound which at low concentrations inhibit the growth of other microbes[8].

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