Journal of ENVIRONMENTAL Treatment Techniques

J. Environ. Treat. Tech.
ISSN: 2309-1185

Journal weblink: http://www.jett.dormaj.com

Effect of cooking on the Climbing Pepper (*Piper nigrum*) on Antibacterial Activity

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Received: 27/12/2015 Accepted: 06/02/2016 Published: 30/03/2016

Abstracts

The effect of cooking on the Climbing Pepper (*Piper nigrum*) on antibacterial activity of climbing pepper or black pepper *(Piper nigrum)* was tested on an *enteric bacterium* (*Escherichia coli)* and on a superficial etiologic agent (*Staphylococcus aureus*) and the activity was compared to the inhibitory activity of 1% Ampiclox (a broad-spectrum antibiotic) as the control. Indication was that the two bacterial test isolates were significantly more sensitive to the hot water, pepper-soup and ethanol extracts of *Piper nigrum* than the Ampiclox control treatment. Results obtained after 72 hours indicated that on the whole, *E.coli* was more sensitive than *S.aureus* in all the treatments. The highest zone of inhibition was shown by the activity of hot water extract on *E.coli* (3.87 E +01 ± 3.8) P = < 0.001. The least activity was by the effect of 1% Ampiclox on *S. aureus* (6.3 E+0 ± 1.7). Pair-wise analysis of the treatments (*Tukey Test*) compared to the effect of Ampiclox on *S.aureus* indicated the following significant responses: Effect of hot water extract on *E.coli* values ranging from 3.87E+01 (± 3.8) to 6.3 E+0 (± 1.7); P= <0.001). Effect of pepper-soup extract on *E.coli* values ranging from 3.66 E+01 (± 2.8) to 6.3 E+0 (± 1.7); P = 0.001. Effect of ethanol extract on *E.coli* values ranging from 3.35E+01 (± 4.2) to 6.3 E+0 (± 1.7); P = 0.003. Effect of pepper-soup extract on *S. aureus* values ranging from 3.14E+01 (± 3.6) to 6.3 E+0 (± 1.7); P = 0.006. The pepper-soup extract thus exhibited significant antibacterial effect against both test bacteria. While the traditional medicinal use of black-pepper from this investigation could be tentatively justified; the probable health implication of the effect of the pepper-soup extract on the enteric and superficial etiologic agents was discussed.

Keywords: *Climbing pepper, Piper nigrum, pepper-soup, Enterobacteriaceace, E.coli, S.aureus.*

1 Introduction

Climbing black pepper has been used for thousands of years to enhance the flavour, colour and aroma of foods and drinks. In addition, they are also known for their preservative and medicinal values [1]. The plant has been shown to have several antimicrobial activities including antibacterial, antifungal and anti-vomiting activities [2]. Antibacterial activity against *Staphylococcus aureus, Bacillus substilis*, and *Candida albicans* was reported by Adams and Moss [3]. Many oils from *Piper nigrum* have been shown to exhibit antimicrobial properties due to the presence of components such as thymol, eugenol, 1-8 cineoles, and β – pinenes [4].

The plant produces an aromatic substance which is added in very small amounts to foods and drinks for the purpose of flavouring and seasoning. From ancient times, black pepper has been used in the treatment of microbial infections [3]. Black pepper is one of the world's most traded spices. The spiciness of black pepper is suspected to be due to the chemical piperine found in the plant. It may be found on nearly every dinner table in the industrialized world, often alongside table salt [4].

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The climbing black pepper *(Piper nigrum*) belongs to the family *Piperaceae*. It is a tropical and subtropical plant family that is widely distributed from India to the rest of the world. Prominent members of this family include numerous fruit trees like the betel *(Piper betal*), long pepper *(Piper longum*), and black pepper *(Piper nigrum*).

Piper nigrum is a climbing vine that grows up to 8-10 m on trees, usually in the forest. Characteristically, the plant grows in a soil that is neither too dry nor susceptible to flooding; that is moist, well drained and rich in organic matter. In humid areas with heavy rainfall, the plant grows in semi-shaded areas and requires support. Fruits which occur in clusters are green when unripe becoming red to red-brown when ripe and black when dry [5, 6].

The plant was introduced to Africa from tropical America [7]. It is a perennial climbing plant cultivated for its fruits in Southern India, South America, and West Indies. This perennial climbing vine is now widely cultivated not only in India but also in many countries like Thailand, Brazil and including Nigeria, Cameroon, Ghana and Benin etc [4].

Green *Piper nigrum* oil contains more oxygenated compounds. The presence of a number of bioactive substances in the essential oil of *Piper nigrum* have been shown; *Piper nigrum* has been reported to exhibit antibacterial activity *in vitro* against some pathogenic microorganisms (which are antibacterial, anti-fungicidal and anti-plasmodia). Further analysis of the extracts of

the fruits revealed the presence of alkaloids and phenolics; these also exert antimicrobial activities [3, 8].

The fruits of climbing pepper *(Piper nigrum*) are economically and medically important. They are popular condiments used as a spicing agent in both Africa and continental cuisines [4]. When ground to powder or in the fruit form, it is used to prepare pepper soup as a stimulant to relieve constipation and control passive uterine hemorrhage in women immediately after child birth. Trado-medically, it has been used to cure mild fever and is known to have diuretic properties. The aromatic stimulating properties of the plant are occasionally used to remove flatulence, correct nausea arising from other drugs, and to allay nausea and vomiting caused by microbes [9].

The fruits and seeds are use by the Ijaws (traditional inhabitants of the Niger Delta region) of Nigeria, as a spice in preparing pepper soup, stew and soup for both the sick and postpartum woman. Also, natives use it as a tonic in the treatment of digestive system dysfunction, rheumatism, nausea, flatulence, laxative, cough, headache, fever, asthma, chronic indigestion, obesity, sinus, congestion, sore throat etc. It could be chewed to reduce sore-throat inflammation; mixed with honey as remedy for night blindness [10]. The present study aims at investigating the effect of cooking pepper–soup (the most popular conduit for using climbing pepper in Nigeria), hot water and ethanol extracts on the antibacterial activity of the climbing pepper fruit.

2 Materials and Methods

2.1 Source of Materials

The dry seeds of climbing black pepper *(Piper nigrum)* were bought at a market, in Amassoma, in Bayelsa State of Nigeria. The seeds were taken to the laboratory and stored at 5 ^{0}C in the refrigerator in order to prevent further dehydration. Identification of the plant was carried out with the aid of the descriptive and pictorial information provided by Nyananyo [5].

2.2 Preparation of ethanol extract

Ten grams (10g) of the pulverized seeds of *Piper nigrum* were ground into fine powder using sterile mortar and pestle. It was then transferred into a beaker containing 100 ml of ethanol and was covered for 24 hours. Whatman No. 1 filter paper was used in a funnel to filter the extract and the ethanol soluble fraction was separated. The filtrate obtained was stored in the refrigerator at 5 ^{0}C for further use.

2.3 Preparation of hot water extract

Ten grams of the pulverized seeds were ground to fine power using sterile mortar and pestle. Ten grams of the powder was soaked in 100 ml of boiled deionized and sterilized water for 24h in a conical flask. This was filtered in a funnel using Whatman No. 1 filter paper. The filtrate obtained was stored in the refrigerator for later use.

2.4 Pepper soup extract

The initial quantity of water used in preparing the pepper soup was 200 ml, other ingredients used alongside with climbing black pepper include: salt, magi, onion, 100g P*iper nigrum, Occimum gratissimum* (scent leaf), crayfish (20g) and dried fish (20g). After preparation, the final quantity of water obtained was 100 ml. This was filtered in a funnel with Whatman No. 1 filter paper and the filtrate was stored in the refrigerator for later use.

2.5 Test bacterial isolates

Test pathogenic bacteria isolates of S*taphylococcus aureus* and *Escherichia coli* used for the tests were obtained from the Department of Pharmaceutical Microbiology Laboratory, Niger Delta University, Bayelsa State of Nigeria. These were isolated from patients and characterized with respect to type and antibiotic susceptibility. The bacterial strains were maintained on nutrient agar slants at 5 ^{0}C.

2.6 Impregnation of filter-paper discs with the extracts

The disk-diffusion technique previously described by Kigigha and Onyema [11], Kigigha and Atuzie [12], Kigigha and Charlie [13] was used to test the antibacterial activity of the different extracts using 10 mm discs. Antibiotic discs were prepared from Whatman No. 1 filter paper using cock borer. The solution containing the extracts (0.1ml each) were impregnated on the disc using sterile pipette and air-dried. A 1% Ampiclox solution (which served as control) was also impregnated on filter-paper discs.

2.7 Antibacterial assessment of extracts

Assessment of the antibacterial activity of the extracts was also carried using the method described by Kigigha and Onyema [11], Kigigha and Atuzie [12], Kigigha and Charlie [13]. Flame-sterilized and cooled pair of forceps the impregnated discs (in triplicates) of the different treatments were placed separately on separate nutrient agar plates with lawn spread growth of the test isolates separately. The filter paper impregnated with 1% Ampiclox solution was also placed separately on the lawn spread test isolates. The discs were well spaced out on the agar plates to avoid overlapping of the zones of inhibition. The plates were incubated at 37°C for 72 hours to observe the zones of inhibition. All treatments were compositely compared as one of the treatments viz. pepper-soup, was prepared according to traditional preparation procedure.

2.8 Statistical Analysis

Data was analyzed using *Zigma-Stat 32* Statistical package. Where normality and homescedasticity were satisfied, data from treatment were analyzed using one-way analysis of variance (ANOVA). Post-hoc *Tukey* tests were used to identify significantly different groups. All the effects were considered significant at the P= 0.05.

3 Results and Discussion

Results obtained after 72 hours (as shown in Fig 1), indicated that on the whole, *E.coli* was more sensitive than *S.aureus* in all the treatments. The highest zone of inhibition was shown by the activity of hot water extract on *E.coli* (3.87 E +01 ± 3.8) mm^2 P = < 0.001. The lowest activity was indicated by the effect of 1% Ampiclox on *S.aureus* (6.3 E+0 ± 1.7) mm^2. Pair-wise analysis of the treatments (*Tukey Test*) compared to the effect of Ampiclox on *S.aureus* indicated the following significant responses: Effect of hot water extract on *E.coli;* values range from 3.87E+01 (± 3.8) to 6.3 E+0 (± 1.7) mm^2; P= <0.001). Effect of pepper-soup extract on *E.coli;* values range from 3.66 E+01 (± 2.8) to 6.3 E+0 (± 1.7) mm^2; P = 0.001. Effect of ethanol extract on *E.coli;* values range from 3.35E+01 (± 4.2) to 6.3 E+0 (±

1.7) mm^2; P = 0.003. Effect of pepper-soup extract on *S.aureus;* values range from 3.14E+01 (± 3.6) to 6.3 E+0 (± 1.7) mm^2; P = 0.006. In Table 1, the test isolates were characterized according to their morphological and biochemical differences.

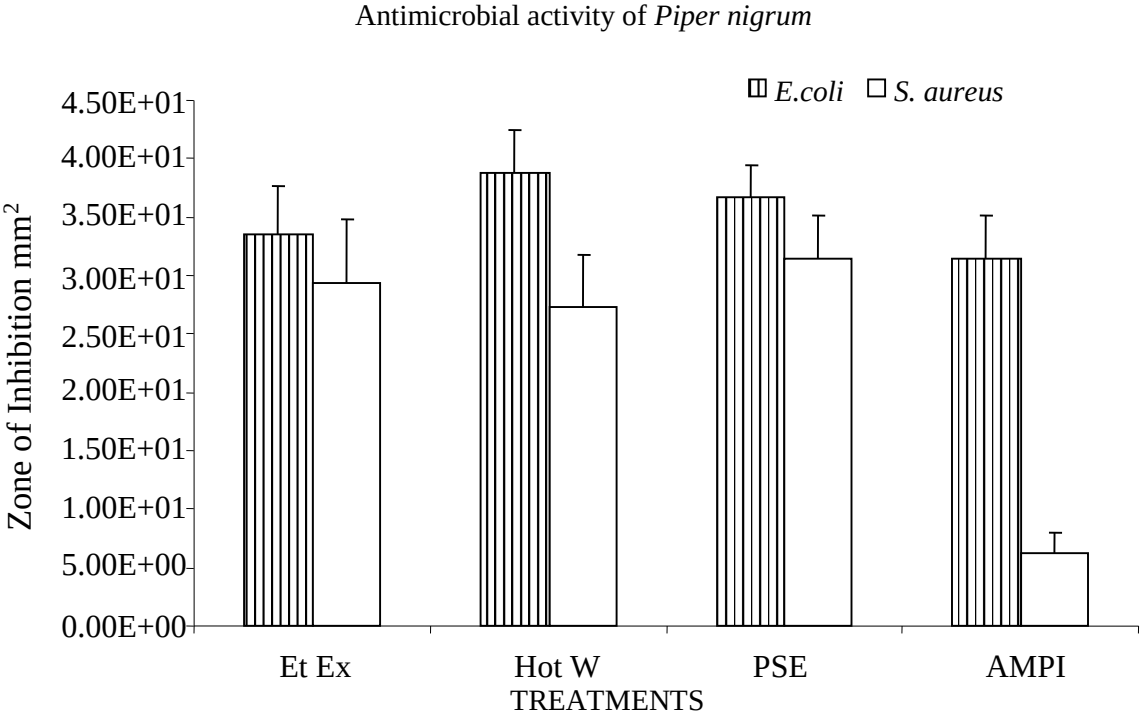

Fig. 1: Antibacterial activity of *Piper nigrum* extracts on *E.coli* and *S.aureus* (P= <0.001). Et Ex = Ethanol extract; Hot. W = Hot water extract; PSE = Pepper soup extract; AMPI= 1% Ampiclox extract. Data = (Mean = ± SE); n = 3.

Table 1: The characterization of the test bacterial isolates

Test and assessment	*Staphylococcus aureus*	*Escherichia coli*
Colony morphology	Pin-point golden	Translucent circular
Gram stain	+ ve Cocci	-ve Rod
Growth in MSA	+ ve	NA
Coagulase Test	+ ve	NA
Catalase Test	+ ve	NA
Sugar utilization	NA	+ ve
Lactose	NA	+ ve
Mannitol	NA	+ ve
Glucose	NA	+ ve
Sucrose	NA	+ ve
EMB	NA	+ ve
CLED	NA	+ ve
KIA Test: H_2S	NA	+ve
Gas	NA	-ve
Butt	NA	Y/Y

KEY
NA = Not applicable
CLED = Represents Cystine-Lactose Electrolyte Deficient
EMB = Represent Eosin Methylene Blue
+ ve = Represents positive reaction
- ve = Represent negative reaction
KIA = Kligler Iron Agar
++ ve = Moderately Resistant
+++ ve = Highly Resistant
R = Resistance

The antibiotic resistance pattern indicated that the test isolates were probably pathogenic as they had acquired some drug resistance from previous contact with the environment. The effect of pepper-soup cooking on the antibacterial activity of *Piper nigrum* was demonstrated; indication was that *E. coli* significantly showed the highest zone of inhibition than *S. aureus* in all treatments group (P= <0.001). Invariably the diverse

use of climbing-pepper in trado-medicine for enteric etiologic agents appears to be justified [9]. Moreover, the fact that the hot-water extract had a higher inhibition zone than the pepper-soup extract appears to indicate decrease in the efficacy of climbing pepper when used in preparing pepper-soup. This could be attributed to the presence of added ingredients (especially of the protenous ingredients such as dried fish, crayfish etc). Interestingly however, the pepper-soup extract indicated significantly higher inhibition (P= 0.006) against *S.aureus* (a superficial etiologic agent) when compared to the effect of 1% Ampiclox on *S. aureus*; and this was the highest inhibition zone by all the treatments on *S.aureus*.

4 Conclusions

This study evaluated the effect of cooking on the Climbing Pepper (*Piper nigrum*) on antibacterial activity of climbing pepper or black pepper *(Piper nigrum)*. The study found that climbing pepper has antibacterial effects against common enteric and superficial etiologic agents. Invariably therefore, there is an indication that climbing – pepper could also find some application in the cure of some yet to be identified infections caused by superficial etiologic agents. More studies would be required to elucidate the entire antibacterial potential of *Piper nigrum* and the several claims of efficacy of this plant on human health improvement and control of disease conditions.

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