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Evaluation of crude and ethanolic extracts of *Capsicum frutescens* var. *minima* fruit against some common bacterial pathogens

Abstract

This study investigated the antibacterial activities of crude and ethanolic extracts of *Capsicum frutescens* var. *minima* fruit against some bacterial pathogens (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aerugionsa*). The pepper was shade dried, blended to powder, and extracted using water and ethanol. Zone of inhibition assessment was carried out using agar well diffusion method. The zone of inhibition for *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* was 14.33 mm, 14.33 mm, 12.00mm and 14.00 mm, respectively for the ethanolic extract, and 11.67mm, 11.33mm, 10.33mm and 12.00 mm, respectively for crude extract. Analysis of variance showed that there was no significantly different ($p>0.05$) among the various bacterium under study for the crude extract and ethanolic extracts. t-test showed no significant variation ($p>0.05$) between crude and ethanolic extracts for each of the isolates except for *E. coli* that showed significant difference at $p<0.05$. The zone of inhibition in both crude and ethanolic extracts of *Capsicum frutescens* var. *minima* suggests that they are potential broad spectrum antibiotics development.

Keywords: *Capsicum frutescens* var. *minima,* Ethanol, Medicinal Plants, microbial pathogens

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Introduction

In many regions of the world, the use of plants as natural spices have increased. This is probably due to their flavor, aroma, colour and nutritional properties. Several plants that are used as food (spices) are also used as medicine. For instance, *Cymbopogon citratus* (lemon grass) and *Zingiber officinale* (ginger), *Myristica fragrans* (nutmeg), *Vernonia amygdalina* (bitter leaf), *Ocimum gratissimum* (scent leaf), *Aframomum melegueta* (alligator pepper), *Piper nigrum* (climbing pepper) are some of plants that are used as medicine and spices for food preparation.[1–7] Typically the use of plant for the treatment of diseases depends on the knowledge about the plant within a given locality. Plants with pharmacological properties abound and many of them are yet to be harnessed due to little information about them.

The use of plants for the treatment of diseases can be trace back to human history. Authors have several reported that a significant number of world population depends on herbs as active ingredients for the treatment of many disease condition.[8–10] The dependency on herbal medicine tends to vary according to level of education and economic factor. Studies have suggested that significant number of individual that patronize traditional medicine practitioners have low source of income and reside in rural areas in many developing nations.[2–7]

Peppers belong to the genus *Capsicum* and family of *Solanaceae.* There over 30 species of the genus *Capsicum*. Chapa-Oliver & Mejía-Teniente[11] reported that the genus Capsicum consists of about 25 wild and 5 domesticated species (including *Capsicum annuum, Capsicum chinense, Capsicum baccatum, Capsicum frutescens* and *Capsicum pubescens*). These domesticated species are the most frequently consumed by humans. Within this species, several taxonomic varieties have formed. This is because domesticated peppers have the tendency to form complexes due to similar morphological characteristics between two or more varieties with regard to their size of fruit, flower appearance. McLeod et al.[12] Jarret[13] have reported overlapping morphological characteristics between varieties of *Capsicum annuum, Capsicum chinense* and *Capsicum frutescens*.

In different parts of the world, pepper is used as medicine and spices. Based on the nutritional properties, pepper have been reported to contain vitamin E and C, and β- carotene. Chamikara et al.[14] reported that *Capsicum* species is an essential spice, flavour enhancer, vegetable and active ingredients in herbal medicine preparation. However, different varieties of pepper are available in different part of the world. Shaimaa et al.[15] reported that *Capsicum annuum* and *Capsicum frutescens* contain vitamin C and E, β-carotene, organic acids (Acetic, Propionic, Succinic, Formic, Butyric Citric, Lactic, Malic and Fumaric), phenolic substance called capsaicin (trans-8-methyl-N-vanillyl-6-non-enamide) and flavonoid at varying stage of their development i.e red (ripe) and green (unripe). The authors also reported varying concentrations of vitamin E and C, β- carotene, organic acid and phenolic substances in each of the pepper varieties. Hot red pepper (*Capsicum annuum*) and chili pepper (*Capsicum frutescence*) is characterized by a pungent smell.[15,16] Basically, the nutrient composition chili pepper is influenced by the maturity stage ripe (red) or unripe (green).[17] According to Bello et al.[16] capsaicinoid alkaloids found in pepper is responsible for the smell.

In Nigeria, five varieties of *Capsicum frutescence* have been reported in literature including maxima, minima, chacoense, baccatum and finger.[16] In a review studies, the genus capsicum have been reported to possess analgesic, hypoglycemic, gastroprotective, anticancer, anti-inflammatory, antidiabetic, antimicrobial, anticholesteremic, anticlotting, antiangiogenic, antiparasitic, anti-arthritic, antineoplastic, antioxidant, antihemorrhoidal, anti-obesity, antipyretic and larvicidal activities,[14,17,18] and provide relief against rhinitis sinusitis, migraine, diabetes and arthritis.[17] Several studies have indicated that different solvents (water, ethanol, acetone) has

varying effects on the zone of inhibition of *Capsicum frutescence* against some common pathogens. Hence this study, aimed at assessing the activities of crude and ethanolic extracts of *Capsicum frutescens* var. *minima* fruit against some bacterial pathogens (*Staphylococcus aureus, E.coli, Pseudomonas aeruginosa* and *Bacillus subtilis*).

Materials and methods

Source of plant materials and extraction processes

The *Capsicum frutescens* var. *minima* fruit was obtained from a smallholder farmer in Ndemili, Delta state, Nigeria. The pepper was further shade dried at room temperature prior to use. The dried pepper was blended to powder. Approximately 30gram of the dried pepper was dissolved in 100ml of distilled water and ethanol separately for 48 hours. Then after the medium was filtered and then concentrated using rotary evaporator.[19]

Source of organisms

The bacterial isolates viz: *Staphylococcus aureus, E.coli, Pseudomonas aeruginosa* and *Bacillus subtilis* used for the study were obtained from Microbiology units, Federal Medical Centre, Yenagoa, Bayelsa state, Nigeria. The biochemical test previously described by Cheesbrough[20] was used to determine the purity of the organisms. Then after, the isolates was inoculated into sterile peptone water and incubated for 24 hours at room temperature prior to use.

Antibacterial activities determination

The zone of inhibition was carried out using agar well diffusion method following the method previously described by Sen et al.[19] with slight modification. Nutrient agar prepared according to the manufacturer's instruction was autoclave and dispensed into sterile Petri dish. About 0.2ml of each of the isolate was spread uniformly on the agar plate using sterilized glass spreader. Sterile cork borer was used to make 6mm wells on the agar plates. 0.3ml of the various plant extracts was dispensed into each well. The plates was well labeled. The Petri plates was incubated at room temperature for 24 hours. The antibacterial spectrum of the extract was determined by measuring the size of clear zone of inhibition across the diameter of the well using meter role.

Statistical analysis

SPSS software version 20 was used to for the statistical analysis. The data obtained was expressed as Mean ± standard deviation. One way analysis of variance was carried at p=0.05 to compare between isolates and Duncan statistics was used to showed source of variation. Furthermore, t-test was used to compare zone of inhibition between the ethanol and crude extract at P=0.05.

Results and discussion

The zone of inhibition of crude and ethanolic extracts of *Capsicum frutescens* var. *minima* fruit is presented in Figure 1. The zone of inhibition of *E. coli* (11.67mm), *Staphylococcus aureus* (11.33mm), *Bacillus subtilis* (10.33mm) and *Pseudomonas aeruginosa* (12.00mm) for the crude extract, being not significantly different at p>0.05 among the various bacterium under study. Furthermore, the zone of inhibition for the ethanolic extract was 14.33mm, 14.33mm, 12.00mm and 14.00 mm for *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, respectively. There was no significant difference (P>0.05) among most of the isolates.

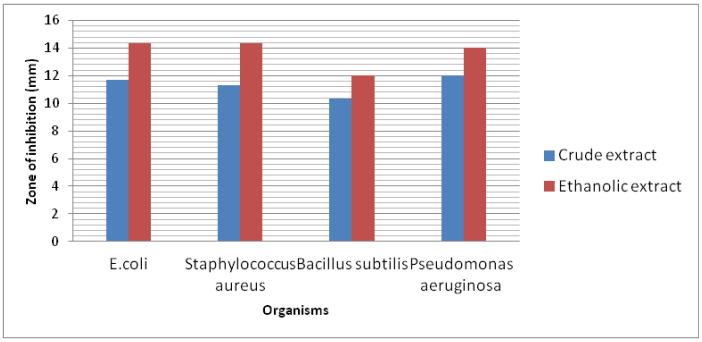

Figure 1 Zone of inhibition (mm) of crude and ethanolic extracts of *Capsicum frutescens* var. *minima* fruit.

The observed variation is between *Bacillus subtilis* and *Pseudomonas aeruginosa*. Based on the comparison of the different isolates (Table 1), there was no significant variation at p>0.05 between crude and ethanolic extract of each of the isolates except for *E. coli*. The bioactive ingredients could account for the pharmacological potentials of pepper including its antimicrobial activities. Bello et al.[16] have reported that 5 varieties of *Capsicum frutescens* viz: *maxima, minima, chacoense, baccatum* and *finger* contain flavonoids, tannins, alkaloids and saponins as active ingredients. Sen et al.[19] reported the presence of alkaloids, tannins, flavonoids, saponins, phenol, carbohydrate, protein, reducing sugar and capsaicin in *Capsicum annuum, Capsicum frutescens, and Capsicum chinense*. Previous studies have reported that flavonoids could account for the antioxidants,[15] anti-bacterial,[21] anti-inflammatory[22] and antiallergic activities.[23] Adefegha & Oboh,[24] Chamikara et al.[14] have also attributed the essential pharmacological potentials (anti-diarrheal, antimicrobial, antioxidant, antihyperglycemic, anti-lithogenic and antimutagenic activities) of chili pepper to the presence of phenolic compounds. Agu & Thomas[25] reported that alkaloids have the tendency to repel pest including microorganisms. Bello et al.[16] noted that alkaloids in pepper could be a contributing factor to its antibacterial characteristics.

Most of the apparent difference that exist between the solvent types, and various organisms could be could be due to the biochemistry, physiology, metabolism and adaptation strategies of the microbes and choice of extraction solvents.[1] The zone of inhibition (for *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*) reported in this study is comparable to the values reported by Sen et al.[19] in *Capsicum frutescens* fruit. Furthermore, the findings of this study also had some similarity in the zone of inhibition of acetone, water and ethanolic extracts of *Capsicum frutescens* var. minima fruit reported by Bello et al.[16] Though, slight apparent variation occurred between the isolates and solvent types. According to Izah,[1] the variations that exist could be due to pH of the medium, temperature, water activity (moisture level), oxygen and nutrient availability, plant biochemistry, age and parts, method of extraction and concentration of the plant extracts. Pundir et al.[17] reported moisture content of 85.700gm and 10.00gm in wet and dry chili pepper.

In addition, both crude and ethanolic extract showed some level of sensitivity to gram negative and gram positive organisms. This suggests that it could be used as a potential broad spectrum antibiotics.[26,27]

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Table 1 Comparative zone of inhibition (mm) of crude and ethanolic extracts of *Capsicum frutescens* var. *minima* fruit

Isolates	Crude extract	Ethanolic extract	t-value	p-value	Implications
E.coli	11.67±0.58	14.33±0.58	-5.657	0.005	Significant
Staphylococcus aureus	11.33±1.53	14.33±1.15	-2.714	0.053	Not Significant
Bacillus subtilis	10.33±0.58	12.00±1.00	-2.500	0.067	Not Significant
Pseudomonas aeruginosa	12.00±1.00	14.00±1.00	-2.449	0.070	Not Significant

Mean± standard deviation (n=3)

Conclusion

Despite the advances made in the field of pharmaceutical microbiology and chemistry, the challenge of antibiotics resistant still exist. Studies have focused on the alternative to synthetic drugs and plant materials have emerged as a credible candidate. Plants have been studied for therapeutic properties probably due the present of bioactive ingredients they possess. *Capsicum frutescens* var. is a notable spices used in some part of the world. But it has been used as active ingredients for herbal medicine preparations in some parts of the world. This study evaluated the antibacterial activities of *Capsicum frutescens* var. *minima* fruit. The study found that both crude and ethanolic extract has antibacterial efficacy. Hence the activities of *Capsicum frutescens* var. *minima* fruit against all the study bacteria viz: *Escherichia coli* and *Pseudomonas aerugionsa* (gram negative) *Staphylococcus aureus* and *Bacillus subtilis* (gram positive) suggests that can be used broad spectrum antibiotics development.

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None.

Conflicts of interest

Author declares there are no conflicts of interest.

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