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Exploring the Biological Potential of the Methanolic Crude Extract of *Capsicum Frutescens* Root

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Abstract

This study examined the biological activity of the methanolic crude extract of *Capsicum frutescens* (C. *frutescens*) root against five microorganisms, three of which were bacteria strains (*Staphylococcus aureus, Eschericia coli and Salmonela typhi*) and the remaining were fungi (*Candida albicans and Aspergillus niger*). The extract of the plant's root was obtained by merceration. The root extract was observed to display significant antimicrobial activity against the microorganisms screened, the highest activity was observed at a concentration of 200 mg/mL. Among the five microorganisms screened, *S.aureus and E.coli* were the most susceptible. The antimicrobial activity decreased as the concentration decreased with *S. typhi, C. albicans and A. niger* exhibiting resistance at the lowest concentration (6.25 mg/mL). Furthermore the root extract was found to contain a significant amount of total phenolic (1.046 ± 0.021 Gallic acid equivalent (GAE mg/g)) and total flavonoid content (0.75 ± 0.25 (RU mg/g)). In addition, the root extract showed a high radical scavenging activity of 91.1% at a concentration of 1 mg/mL, indicating the root's potential antioxidant properties. The results of this work reveals that the methanolic crude extract of the *C. frutescens* root is a potential natural source of antimicrobial agent with the phenolic and flavonoid content potentially contributing to its activity. Further research into the isolation of the active principles in *C. frutescens* root is vital to ascertain the individual principles responsible for the biological activity in this plant part.

Keywords: Capsicum frutescens, bioactivity, bacterial, fungi, antioxidant

INTRODUCTION

The use of medicines with plant origin in place of the synthetic alternatives has now been greatly adopted globally. This is because they are readily available in nature, free and have fewer or no side effects compared to the synthetic ones. Nature herself has diverse medicinal plants with varying medicinal properties. *C. frutescens* is one of such plants that nature has blessed man with because of its diverse biological potentials. C. frutescens a species of the chilli pepper which originates from the tropical and humid regions of the Central and South America (Menichini et al., 2009, Ibiza *et al.,* 2012) is a ubiquitous plant and is widely employed as food flavoring agent, coloring agent, additive to livestock feed and in food and medicinal application (Olatunji and Afolayan, 2019, Bagwai *et al.,* 2022).

The potential of *C. frutescens* in medicinal application is due to the presence of numerous non-nutritive but biologically active compounds known as phytochemicals (Antonious *et al.*, 2006). Among the biological activities displayed by these compounds (phytochemicals) is the ability to reduce oxidative stress, neutralize free radical as well as repair cell damage. These aforementioned activities are actualized by the plants as a result of their antioxidant potential (Batiha *et al.*, 2020).

The phenolic components of the phytochemicals have been found to be the largest category of the phytochemicals (King and Young, 1999) that can be said to be the driving force behind

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the activity of the plants due to their antioxidive nature (Shahidi, 2000).

RELATED WORKS

Several reports on the phytochemistry of the different parts of *C. frutescens* have been published in the literature. Gurnani *et al*., (2016) worked on the *C. frutescens* seeds, its chemical composition, antimicrobial and antioxidant potentials were explored, and they found that the seeds possessed numerous biologically active volatile compounds. Their worked also showcased the remarkable inhibitory activity of the plant against selected disease-causing microorganisms.

A study comparing the phytochemical contents and the antioxidant activity of *C. frutescens* and Capsicum annuum L. by Olatunji and Afolayan (2019) also revealed the presence of huge amounts of phytochemical contents and remarkable antioxidant activity indicating its pharmacological and nutritional value. The potential of *C. frutescens* fruit extract to prevent fungal infection during groundnut storage has also been explored. The leaf and fruit extracts were studied for their activity against *Aspergillus flavus, A. niger, Penicillium sp.* and *Rhizopus sp.* and the extracts from the two plant parts showed strong inhibitory activity against *Aspergillus flavus* (Soumya and Nair, 2012). A recent review showcased the different bioactive compounds that have been isolated from *C. frutescens* to include phenolic compounds, alkaloids, flavonoids, essential oils, esters, hydrocabons, steroids, tanins as well as capsaicin-related compounds, to mention a few (Batiha *et al.*, 2020).

Despite the numerous reports on the different parts of *C. frutescens*, to the best of our knowledge, this report is the first on the root of *C. frutescens*, providing information on the components as well as the biological activity of *C. frutescens* root. Going by this, the quantification of the phenolic content and flavonoid content as well as its antioxidant activity was carried out in this study. The antimicrobial activity of the root extract was also tested against five different microorganisms.

MATERIALS AND METHODS

Sample Collection

Roots of *C. frutescens* were obtained from Lacot farm, Odoyanta, Ijebu ode, Ogun state, Nigeria.

Preparation of Extracts

The roots were air dried and cut into small bits before being ground into fine powder in a mechanical blender. The dried root powder was transferred into airtight storage vials and stored in a cool dry place pending further analysis.

Determination of Phenolic Content

0.02g of the sample was weighed and dissolved in 100 mL of distilled water followed by 0.2 mL of Folin C in a conical flask. 15 % sodium carbonate (1 mL) and distilled water (2 mL) was then added to the mixture. The mixture was allowed to stand for two hours till a blue coloration was observed this was then read on the UV spectrometer at a wavelength of 765 nm. The total phenolic content was calculated from the calibration curve and the results were expressed as mg of Gallic acid equivalent per g dry weight.

Y = 0.0981x – 0.0099 (gallic acid equivalent),

Where Y = absorbance

$$Total\ phenolic\ content = \frac{X\ x\ Total\ volume}{Mass\ of\ crude\ sample} \qquad (1)$$

Determination of flavonoid content

2 % HCl (50 mL) was added to 5 g of *C. frutescens*, in a beaker and then boiled on a water bath for 30 minutes. The boiled mixture was cooled and then filtered. A portion of the filtrate was taken, and an equal amount of ethyl acetate was added to it beginning with drop to obtain a precipitate. The precipitated flavonoid was filtered using a weighed filter paper. The precipitate on the filter paper was then oven dried and the filter paper was reweighted after oven drying to obtain the mass of the dry flavonoid.

$$Flavonoid\ (\%) = \frac{W_2 - W_1}{W_o}\ x\ 100 \qquad (2)$$

Where W_o = Weight of Dry Sample

W_1 = Weight of Empty filter paper

W_2 = Weight of Filter paper and precipitate after oven drying

Antioxidant Activity

The radical scavenging activity assay of *C. frutescens* root extract was evaluated using the DPPH assay method of Brand-Williams *et al* with minor modification. 1 ml of the extract was added to 1 ml of 0.2 mmol of DPPH (2 2'-diphenyl-1-picrylhydrazyl) in methanol solution. The mixture was then allowed to stand in the dark at room temperature for 30 minutes. The absorbance of the extract and the standard were then taken at 517 nm. The percentage free radical scavenging activity was then calculated:

$$\%\, Scavenging\ activity = \frac{[A_o - A_1]}{A_o}\ X\ 100 \qquad (3)$$

Where A_0 is absorbance of the control (blank sample) and A_1 is absorbance of extract.

Antimicrobial Screening

The crude extract of *C. frutescens* was tested on three different bacterial strains (*S. aureus, E. coli, S. typhi*) and two different fungal strains (*C. albicans and A. niger*). The microorganisms were cultured in a Thermo Scientific (Waltham, MA, United States) Oxoid Nutrient agar (NA) at 37 °C for 24 hours. The disc diffusion method (Ha et al., 2019) was used to determine the antimicrobial activities of the crude extract. Petri plates were prepared by pouring 20 mL Thermo Scientific Oxoid Nutrient agar (NA) in them and the solution was allowed to solidify. The plates were then dried, and 0.1 mL of the standardized inoculum containing 106-107 colony-forming units/mL of the bacterial suspension was poured, uniformly spread, and allowed to dry for 5 minutes. The crude extract was prepared in methanol which served as the negative control. 100 µL was taken from this stock solution and was added to respective wells. Only methanol (100 µL) was introduced into the control well. Gentamycin (positive control) was used as the reference antibiotic for bacterial and Tioconazole was used as the reference antifungal for fungi. The plates were left at room temperature to allow diffusion take place and then incubated at 37 °C for 24 hours to allow microbial growth. The antimicrobial screening was determined by measuring the diameter of the zones of inhibition against the test organisms. The experiments were repeated in triplicate and the results are expressed as mean values.

RESULTS AND DISCUSSION

Antimicrobial activity

The results of the antimicrobial activity of the methanolic crude extract of *C. frutescens* root examined against five different pathogenic microorganisms (three bacteria and two fungi) are presented in Table 1. The extract displayed significant activity (≥ 10 mm) against almost all the microorganisms screened at concentrations between 12.5 to 200 mg/mL. The best activity of the extract was observed at a concentration of 200 mg/mL with significant activity (relative to the positive control) because a high zone of inhibition was recorded at this concentration. A zone of inhibition of 26 mm was recorded against *S.aureus*, 24 mm against *E. coli*, 20 mm against *S. typhi*, 18 mm against *C. albicans* and 18 mm against *A. niger*. Subsequently, the activity of the extract dropped as the concentration reduced. At the lowest concentration (6.25 mg/mL) three (*S. typhi, C. albicans and A. niger*) out of the five microorganisms were resistant to the effect of the plant extract while *S. aureus* and *E. coli* were the most susceptible to the effect of the crude plant extract regardless of the concentration. The positive control (Gentamycin (10 mg/ mL)) displayed better activity while the negative control (methanol) was not active against any of the organism hence indicating that the solvent had no additive effect on the activity of the plant extract.

Table 1. Antimicrobial activity of crude extract of *C. frutescens* root (inhibition zone in mm)

Conc (mg/mL)	Bacterial			Fungi	
	S.aureus	**E. coli**	**S. typhi**	**C. albicans**	**A. niger**
200	26	24	20	18	18
100	22	20	17	16	14
50	18	17	15	14	12
25	14	14	13	12	10
12.5	12	12	11	10	10
6.5	10	10	0	0	0
+ ve	40	38	38	28	28
- ve	0	0	0	0	0

Values are expressed as the mean of three separate experiments. +ve control for antibacterial activity: Gentamycin (10 mg/ mL) and for antifungal: Tioconazole (70 %)

Though no preliminary phytochemical screening was carried out in this work or mechanism of action studied, but previous studies on the *C. frutescens* plant as revealed by various researchers shows that all plant parts are highly rich in phytochemicals some of which are alkaloids, flavonoids, phenolic and proanthocyanidins among others (Oboh and Ogunruko, 2010, Zhuang *et al.,* 2012, Nascimento *et al.,* 2014, Gurnani *et al.,* 2016, Keser *et al.,* 2018, Afolayan and Olatunji, 2019). These compounds have been proven to possess pharmacological properties which may be responsible for the different biological activity of the root extract. Recently research direction is focused on substituting toxic synthetic drugs principles with natural ones thus *C. frutescens* can be said to be suitable source of natural medicinal principles able to function in this respect as it is ubiquitous. Previous studies have shown *C. frutescens* to elicit numerous antimicrobial effects.

C. frutescens was reported to be potent against 16 different fungal strains by rupturing the membrane integrity of the cells (Careaga et al., 2003, Soumya and Nair, 2012, Batiha, et al., 2020), its fruit seed powder was also shown to regulate *Callosobruchus maculatus* (F) in stowed cowpea as well as *Sitophilus zeamais* in stowed maize (Ileke et al. 2013). In 2015, Bello *et al* reported the activity of *C. frutescens* at different concentrations (250 mg/mL, 200 mg/mL, and 150 mg/mL) and showed that the extract of the fruit was very active against all the bacterial strains screened with a zone of inhibition ranging from 13.66 mm to 22.33 mm. In another work, Chrysoeriol a phenolic compound was found to be the most active compounds against seven different microorganisms (Nascimento *et al.,* 2014).

The potential of the root extract to inhibit fungicidal activity also corroborates the work of Soumya and Nair, 2012 who showed that the extract of the fruit and leaf had strong inhibitory activity against *A. niger* and *A. flavus*. Going forward, the broad-spectrum antimicrobial activity exhibited by the methanol extract of *C. frutescens* root in this study confirms its medicinal and preservative potential against infectious and food borne pathogens. The significant antimicrobial activity may therefore be attributed to the presence of the phenolic compounds present in it. Reports have shown that the presence of phenolic and flavonoids compounds in pepper makes it capable of anti-inflammatory activity (Gurnani et al., 2016, Muthuswamy, et al., 2021).

Based on the aforementioned the root of *C. frutescens* may be said to be a rich source of phenolic compounds that are vital phytochemicals in many plants which display physiological ability and is thus responsible for their medicinal potentials.

Table 2. % DPPH scavenging activity, Total phenolic content (TPC) and total Flavonoid content (TFC) of crude extract of *C. frutescens* root

Activity	Value
% DPPH scavenging activity	91.1 %
TPC	1.046 ± 0.021
TFC	0.75 ± 0.25

The TPC and TFC was found to be 1.046 ± 0.021 Gallic acid equivalent (GAE mg/g) and 0.75 ± 0.25 (RU mg/g) respectively (Table 2). Phenolics and flavonoids are important plant secondary metabolites that display antioxidant potential as a result of their ability to donate hydrogen (Gurnani et al., 2016) as well as prevent the conversion of hydroperoxides into free radicals (Fatemeh et al., 2012, Saeed *et al.*, 2019), chelate metal ions, and enzyme inhibition (Nurhaslina et al., 2019).

The phenolic content was found to be more abundant than the flavonoid content in the plant's root. This observation is in resonance with related studies involving other parts of the plant. With different extraction solvents, a very significant amount of phenolic was isolated from different parts of the plant in the range of 3.2 ± 0.22 to 110.6 ± 1.03 by Nascimento *et al.*, in 2014. Gurnani et al., (2016) also found the phenolic content to be an abundant secondary metabolite of the *C. frutescens* seeds besides the flavonoid content. The pericarp of *C. frutescens* fruit as well is a natural source of abundant amount of phenolic (Oboh and Ogunruko, 2010). The phenolic and flavonoid content in this present study is however lower in the plant part used (root) compared to the quantities that have been reported for other part in other studies (Nascimento, *et al.*, 2014, Gurnani *et al.*, 2016, Keser et al., 2018, Olatunji and Afolayan (2019). This disparity can be attributed to biotransformation which may have taken place during translocation of the phytochemicals from the root to other plant parts, including the fruit, thus causing an increase in the plant chemicals in these other parts. However, a related study on the root of *Capsicum chinense*, another chilli species, revealed the presence of large amount of phenolic compounds in the roots compared to the leaves and suggested its presence to be responsible for providing mechanical strength and protection against pathogenic attack at this region (Emanuel et al, 2019).

Antioxidant Activity by DPPH Assay

The ability for total phenolic and total flavonoid content to complement DPPH radical scavenging activity makes DPPH assay a suitable method for determining the antioxidant activity in this work. DPPH assay is a known and accurate method employed in the antioxidant activity determination. The antioxidant activity while using DPPH radical scavenging is usually carried out at ambient temperature. This strikes out the possibility of molecular degradation which may arise as a result of heat. This thus makes it a preferred method for antioxidant assays (Bondet *et al.*, 1997).

The root extract of the plant showed a high DPPH radical scavenging activity of 91.1 % at a concentration of 1 mg/mL. The radical scavenging activity correlates with reports of other researcher who worked on different parts of the plant, indicating that *C. frutescens* exhibits a high radical scavenging ability regardless of the plant part screened. This high scavenging activity was displayed when Otunla and Afolayan (2013) observed high DPPH radical scavenging activity with the aqueous extract of the fruit. Oboh and Ogunruku also reported an DPPH radical scanvenging activity of 73.7 %. Keser et al 2018 also observed a similar trend while using water and ethanol extract. When they screened the fruit of *C. frutescens* for its antiradical activity using three radical scavenging test method (ABTS, OH- and DPPH) they found that the radical scavenging activity were all high in the test with the ethanol extract, however, the DPPH scavenging activity gave better result with the water extract (ethanol 69.89 %, water: 84.14 %). This observation from other researchers who used other plant parts as well as the result from the present study on the plant's root brings to light

the potential of *C. frutescens* to be a good source of antioxidant.

CONCLUSION

The present study was carried out to screen *C. frutescens* root for its biological potential. The result of the experiment reveals that the methanolic crude extract of the *C. frutescens* root is a potential natural source of antimicrobial agents with the phenolic and flavonoid content potentially contributing to its biological activity. The methanolic root extract of *C. frutescens* displayed broad spectrum antimicrobial activity, and the root extract showed a high radical scavenging activity.

The presence of biologically active compounds, alongside the broad-spectrum antimicrobial activity as well as the remarkable antioxidant activity of the root of *C. frutescens* in the present study makes it a potential medicinal source in tradition medicine preparations as well as in food preservation. These findings can serve as a foundation for future research into the usefulness of this part of the plant that has been perceived as a mere food waste. Isolation of the active principles in *C. frutescens* root can offer solution to the issue of prevalent antimicrobial resistance.

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