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DETERMINATION OF TOTAL PHENOLIC CONTENTS FROM *Capsicum frutescens* (RED PEPPER) FRUITS EXTRACTS

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ABSTRACT
The study aimed to extract* Capsicum frustecens *fruits (Red pepper) and determine the total phenolic contents in the crude extracts. The methodology involved using aqueous and ethanol as solvents, and both were extracted for 48 and 72 hours, respectively, at room temperature. Filtrates were subjected to evaporation in a rotary evaporator. Ethanol had recovered the highest extract of 13.43g (26.04%), while aqueous extract had recovered 09.52g (19.4%). Total phenolic contents of the* Capsicum frustecens *fruits were determined by Folin Ciocalteau method, standard gallic acid solution was prepared in 100ml of ethanol to produce a stock solution. Various concentrations of working solutions (20, 40, 60, 80, 100 µg/ml) were prepared. Samples were prepared in triplicates for each assay, and a mean absorbance value was recorded at 760 nm in a UV spectrophotometer. The results showed that* Capsicum frustecens *ethanolic extract had recorded the highest mean total phenolic content of 113.79±1.63 mg GAE/g than aqueous extract recorded 78.33 mg GAE/g. The highest phenolics content in ethanolic extract was (196.77 mg GAE/g) recorded in 100µg concentration, while the lowest phenolic content (29.33 mg GAE/g) was recorded in 12.5µg concentration of aqueous extract. The total phenolic contents showed no significant difference (p>0.05) among the extracts. The results obtained from the study highlighted the potential of* Capsicum frustecens *fruits from the plant-based origin as a promising source of phenolics and other phytochemicals such as flavonoids, alkaloids, etc., in food and pharmaceutical industries.
Keywords: Extracts, Phenolics, Phytochemicals, Reagent, UV spectrophotometer.

INTRODUCTION

The genus *Capsicum* belongs to the *Solanaceae* family (Bosland and Votava, 2000), in West Africa, the genus is represented by two cultivated species viz: *Capsicum annuum* and *Capsicum frutescens*, with numerous varieties that are closely related morphologically (Olatunji and Afolayan, 2019). Farmers in the tropical and subtropical regions grow *Capsicum* spp., for its economic benefit due to its uses in food as spices or its importance in high nutritional values (Dimitros, 2000).

According to Abdelraouf *et al.* (2014) red pepper has been added to foods since ancient times to improve food delicacy, preservatives, or as folk medicine. The dried and grounded *C. frutescens* fruits produce the red pepper in a powdery form, often used as a food additive (spice) to confer aroma, colour different types of foods, or treat various human ailments (Gurnani, *et al*., 2015). Chandra *et al*. (2014) reported that red pepper has been used as an alternative medicine for the treatment of some ailments. Red chilli provides a rich source of bioactive compounds to confer nutritional and health benefits (Olatunji and Afolayan, 2019). According to Gurnani, *et al.* (2015), the substances responsible for the pungency of *C. frutescens* pods are the capsaicinoids alkaloids, which are known for their pharmacological, neurological and dietetic effectiveness.

Plant secondary metabolites contain phenolic compounds, and their presence in plants confers numerous potential benefits, (Chandra *et al*., 2014). Phenolic substances belong to the category of phytonutrients that exhibit huge antioxidant activities. Phenolics can be divided into simple phenols, flavonoids, hydroxycinnamic, and phenolic acid. Numerous studies have reported that phenolic substances act as potent antioxidant components (Saeed *et al*., 2019).

The purpose of this study is to investigate the total phenolic content in *Capsicum frustecens*, because there is limited information and research on total phenolic content in common plant-based foodstuff *Capsicum frustecens* inclusive.

MATERIALS AND METHODS

Sample Collection

The dried fruits of *Capsicum frustecens* were purchased from the retailers in Janguza market of Tofa Local government, Kano state, Nigeria, collected in a black polyethylene bag, and conveyed to the laboratory for the study. *Capsicum frustecens* fruits were confirmed and duly authenticated with accession number (BUKHAN 0468) by comparison with a voucher specimen kept at the herbarium of at Department of Plant Biology, Bayero University Kano by a Botanist (Bukar *et al.*, 2010).

Preparation of the sample

The *Capsicum frustecens* fruits were rinsed with distilled water to remove dust and other impurities, placed under the flow of air until completely dried, then ground into a powder form using an electrical blender, conveyed in a transparent plastic container, covered and labelled accordingly, and stored in a refrigerator at 40C before the extraction (Azees *et al.*, 2012).

Extraction

Extractions were performed according to the procedure described by Rahiman *et al.* (2013) as about 50g of the samples was weighed and soaked in two labelled conical flasks containing 500ml each of aqueous and 90% ethanol, respectively, the former extracted for 48 hours while the latter for 72 hours at room temperature with thoroughly shaking at 9 hours intervals. The solutions were filtered, then evaporated in a rotary evaporator, recovered crude extracts were weighed, and their properties were observed. Extracts were stored in the refrigerator until further analysis (Bagwai *et al.,* 2019).

Total Phenolics Content

Total phenolics content was determined by Folin Ciocalteao method as described by Asli *et al*. (2010) and Genwali *et al.* (2013) with minor modifications. The standard gallic solution was prepared as about 25mg of gallic acid was dissolved in 100ml of ethanol to produce different concentrations of stock solutions (20, 40, 60, 80, 100 µg/ml). Furthermore, to produce working stock solutions, about 0, 1, 2, 3, and 4mls aliquots were pipetted into a 10ml test tube and then subsequently diluted to volume with ethanol. A standard curve was generated by adding 1ml of Gallic acid into a test tube followed by 5ml Folin reagent. After 5 minutes, 4mL of Na_2CO3 was also added, a blue coloured solution was produced, the mixture was shaken thoroughly and incubated in the dark at room temperature for about 1 hour. Finally, a UV spectrophotometer recorded mean absorbance from the triplicate analysis at 760 nm. Furthermore, absorbance for the extracts was recorded after following a procedure as described for the standard solution/gallic acid. Total phenolics compounds were determined from the regression equation of the standard calibration curve (Y=mx+b, R2= 0.985) and expressed as mg GAE/g in dry weight (mg/g).

Statistical analysis

All the assays were performed in triplicates, and results were recorded three times and expressed in mean and ± standard deviation. Data were evaluated by analysis of variance (ANOVA) at $p<0.05$ level of significance.

RESULTS AND DISCUSSION

The study showed ethanol to yield the highest extraction (13.43g) compared to the aqueous that recovered (09.52g). This result was also in accordance with the findings of other researchers like Bello et al. (2015), who reported that ethanol recovered the highest extract yield among the other solvents, and Bukar, (2012), who said ethanol possessed more substantial extraction capacity as a solvent. The result also contradicts the finding of Nguyen *et al.,* (2015) aqueous extract recovered the highest yield, also Olatunji and Afolayan (2019) reported the highest extraction yield in aqueous extract compared to the ethanolic extracts from *Capsicum* species. Therefore from the study, ethanol was the best solvent for the extraction of the *Capsicum frustecens* as the yield of extraction is related to the strong affinity of the solvent.

Table 1: Physical characteristics of the extracts

Properties	Aqueous	Ethanolic
Weight (g)	50	50
Solvent (ml)	500	500
Recovered (g)	09.52	13.43
Recovered (%)	19.4	26.08
Colour	Blue-green	Orange
Texture	Sticky	Oily

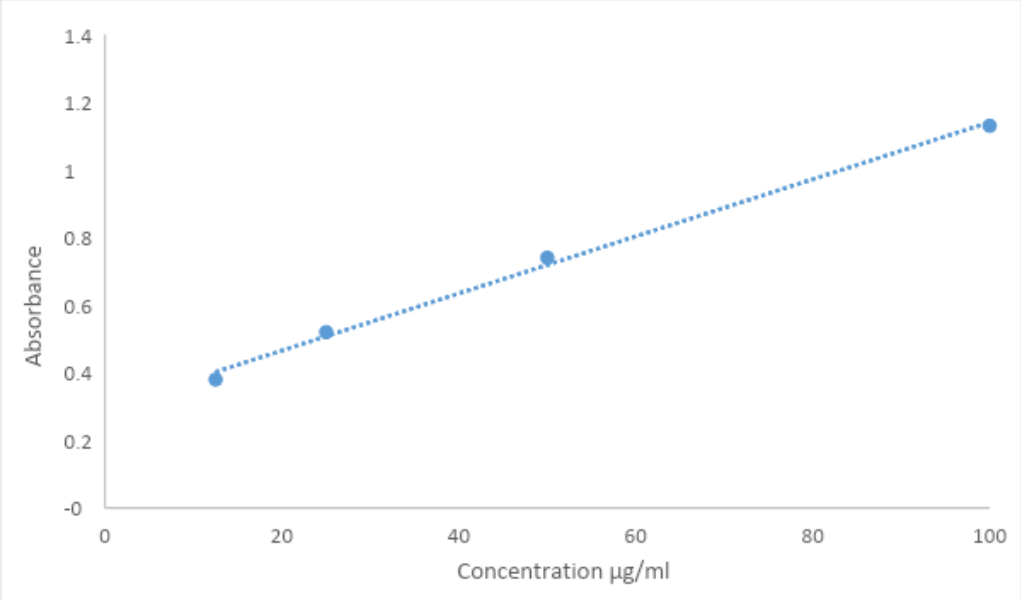

Fig 1. Calibration curve for standard gallic acid

Table 2: Total Phenolic content in different concentrations

Concentrations (µg/ml)	Aqueous (mg GAE/g)	Ethanolic (mg GAE/g)
100	145.33±2.86	196.77±1.63
50	83.66±1.69	142.53±1.63
25	54.33±2.05	78.35±1.24
12.5	29.33±2.05	37.54±1.63
Mean	78.33	113.79

The results are expressed as mean ± standard deviation of triplicate results, p>0.05

The mean total phenolic content of *Capsicum frustecens* fruit ethanolic extracts discovered was 113.79 mg GAE/g, while aqueous was 78.33 mg GAE/g. The highest phenolics content in ethanolic was recorded in 100µg concentration (196.77 mg GAE/g) while the lowest phenolic content was 29.33 mg GAE/g recorded in aqueous as shown in Table 1. The results of the study recorded higher phenolic content from the ethanolic extract; the difference in the impact of the phenolic content from both the ethanol and aqueous might be from the different extraction abilities. This study agrees with Akeem et al. (2016), where a similar observation was reported as *Capsicum frustecens* reported higher phytochemical contents in the ethanolic extracts. A previous study by Azees *et al.* (2017) recorded 200 mg GAE/g of phenolic content from *Capsicum frustecens*. It has also been reported by Saeed *et al* (2019) that *Capsicum spp.* had the highest total phenolic contents (213±1.24 mg GAE/g) among the 12 vegetables observed. The equation of the standard curve is y = 0.0114x + 0.2365, R2=0.997 (Fig. 1). Total phenolics contents showed no significant difference (p>0.05) among the extracts but were significantly higher than the aqueous. It is well-known that phenolic compounds contribute to the quality and nutritional value in modifying colour, taste, aroma, and flavour and providing beneficial health effects (Rahiman *et al.,* 2013). The *Capsicum spp* fruit confers a range of bioactive phytochemicals, including flavonoids, carotenoids, phenolics, and other antioxidant compounds (Dimitros, 2000). Moreover, various factors affect phenolic content, such as variation of climatic conditions, soil sensitivity, etc. (Saeed *et al.,* 2019).

CONCLUSION

Observations based on the study result showed *Capsicum frustecens* is a good source of phenolic compounds; therefore, the remarkable amount of the total phenolic content might possess antioxidant properties, leading to novel research in the future.

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