Isolation and quantification of phenolic constituents in Pepouri for pharmaceutical applications

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Abstract

Cucumis p. is a widely cultivated vegetable crop, particularly grown in Southern Africa and other regions, including Nigeria. Its fruits, flowers, and seeds are commonly consumed, either raw or cooked. This species is nutritionally rich, containing essential compounds such as calcium, iron, proteins, oils, and vitamins. Notably, it provides vitamin A and natural antioxidants, including vitamin C, vitamin E, and β-carotene, which help protect sensitive biomolecules from oxidative damage and delay the aging process. The total phenolic content of *Cucurbita p.* was determined using the Folin–Ciocalteu reagent. Among the different extracts, the petroleum ether fraction exhibited the highest phenolic content (17.1 mg/g gallic acid equivalent), followed by the methanol extract (12.6 mg/g), while the ethanol extract showed the lowest value (8.0 mg/g). HPLC chromatographic analysis further confirmed the presence of bioactive compounds, with distinct peaks observed at 254 nm at varying retention times.

These findings suggest that *Cucurbita p.* possesses significant antioxidant activity, highlighting its potential as a natural source of antioxidants.

Keywords: Cucurbita plant sample, antioxidant, Phenolic contents, Lowry solutions, Gallic acid solution

1. Introduction

Cucurbita pepo is a plant cultivated widely in southern Africa and other regions of the continent, including Nigeria. Belonging to the family Cucurbitaceae, it is valued for its multiple edible parts—leaves, fruits, flowers, and seeds—which can be easily prepared and consumed. According to Smith (1997), it is one of the most important plants used for food in these regions. *C. pepo* is rich in essential nutrients, including calcium, iron, vitamins, oils, and proteins. In western Moshna Land, Zimbabwe, its leaves serve as a staple vegetable, consumed on average 3.9 times per week during the rainy season (Dersluijer et al., 1997). The plant thus provides a significant source of nutrients required for human health. Pumpkin seeds are also widely consumed as snacks in several countries, such as Greece, either raw or roasted, and are often incorporated into bread, muesli, salads, and cakes. Moreover, gourd seed oil is not only a popular edible oil but also recognized as a dietary supplement, offering multiple health benefits as a natural source of protein (Phillips, Ruggio, et al., 2005). Additionally, cucumber seed oil has demonstrated hypoglycemic effects, suggesting its potential role in alleviating diabetes (Caili et al.).

1.1 Nutritional values of *Cucurbita pepo*

Cucurbita pepo is a widely cultivated vegetable valued for its rich content of essential antioxidants and vitamins. Despite being a low-calorie food, the commonly grown varieties are abundant in natural polyphenolic and flavonoid compounds such as lutein, zeaxanthin, and β-carotenes. These carotenoids serve as important precursors of vitamin A within the human body (Yadav et al., 2010).

1.2 Ethnomedicinal uses

Members of the Cucurbitaceae family are extensively utilized in traditional medicine. Different species are employed in the treatment of burns, abrasions, respiratory disorders, skin diseases, and inflammatory conditions, and they also serve as diuretics and medicinal essences (Gilani et al., 2005; Mukherjee and Wahile, 2006). The seeds of *C. pepo* are particularly rich in proteins, magnesium, calcium, and potassium. Traditionally, they have been used as anthelmintics, aiding in the expulsion of intestinal worms, tapeworms, and pinworms from the human body. Furthermore, regular consumption of *C. pepo* contributes to the prevention of cardiovascular diseases, diabetes, and certain types of cancers (Aiyegoro, 2010).

1.3 Antioxidants

Antioxidants are chemical compounds that neutralize free radicals—atoms or molecules with unpaired electrons that arise naturally during metabolic processes. These free radicals, while useful in certain physiological reactions, can also cause oxidative stress when present in excess. Secondary plant metabolites such as phenolic acids, polyphenols, and flavonoids are water-soluble compounds with potent antioxidant properties, protecting plants and, subsequently,

consumers from oxidative damage (Moon and Shibamoto, 2009). In humans, dietary antioxidants reduce the risk of chronic diseases such as cardiovascular disorders, diabetes, and cancer.

C. pepo is an excellent source of natural antioxidants including vitamins A, C, and E, as well as β-carotene, which collectively prevent free radical-induced damage to biomolecules and slow the aging process. Antioxidant-rich foods such as fruits and vegetables are frequently highlighted for their health-promoting effects (Omenn et al., 1996).

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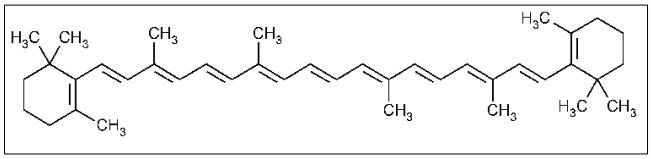
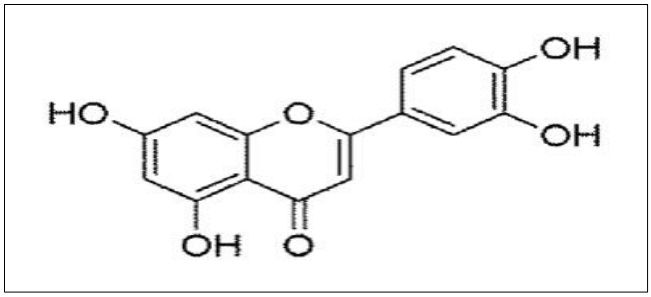


Beta Carotene



Luteolin

Fig 1: Some antioxidant compounds isolated from plants

The leafy vegetable derived from *Cucurbita pepo* is among the most widely consumed and palatable food sources in southwestern Nigeria. Vegetables play a vital role in the human diet, particularly in developing countries, as they provide essential vitamins and minerals often lacking in staple foods. Compared to cereals such as rice, vegetables yield higher amounts of nutrients per unit of cultivated land (AVRDC, 1990). The beneficial health effects of fruits and vegetables have been largely attributed to their high antioxidant content (Ames et al., 1993; Evans & Miller, 1995). Antioxidants, naturally present in fruits and vegetables, are micronutrients with the capacity to neutralize free radicals (Cadenzas & Packer, 1996; Nicoli et al., 1999). Free radicals, in turn, have been implicated in the onset of several chronic diseases, including cancer, cardiovascular disorders, and diabetes (Sies, 1996; Yoshikawa et al., 2000; Devasagayam et al., 2004).

1.4 Aims and Objectives

The present study aimed to evaluate the antioxidant potential of extracts from *Cucurbita pepo* in comparison with black tea using DPPH radical scavenging activity and total phenolic content assays. In addition, the study sought to identify the HPLC profiles of compounds present in *Cucurbita pepo*.

Plate 1: *Cucurbita pepo* plant

2. Materials and Methods

2.1 Materials, Apparatus, and Equipment

The instruments used included a weighing balance, HPLC system, UV lamp, IR spectrophotometer, and UV-visible spectrophotometer (Instruments Laboratory). Analytical grade solvents such as ethanol, chloroform, methanol, and petroleum ether were obtained from the departmental store or purchased from Sigma-Aldrich. Distilled water was supplied by the Analytical Laboratory. DPPH, gallic acid, Folin–Ciocalteu reagent, sample bottles, vials, and powdered leaves of *Cucurbita pepo* were provided by Dr. Ahmad A. Yakasai (supervisor).

2.2 Extraction

A total of 100 g of powdered plant material was percolated in ethanol for seven days with continuous shaking. The mixture was filtered, concentrated using a rotary evaporator, and air-dried under a fan for three days. The dried sample was weighed and labeled as the crude extract. Further fractionation of the crude extract was performed using a separating funnel with solvent systems of different polarities: chloroform/water (1:1) and petroleum ether/aqueous methanol (1:1). The resulting extracts were concentrated, dried, weighed, and labeled as F5 and F4, respectively.

Scheme 1: A chart of extraction processes.

2.3 DPPH Assay

2 mg of each extract was weighed into a vial and 2 mL of methanol was added to bring the concentration to 10 mg/mL (10,000 µg/mL).This solution was labeled as stock solution. Serial dilution was employed for the preparation of the test solutions (1000-10µg/ml), using methanol. 0.1ml of the test sample was a liquated in a 96 well plate and 0.2ml of the prepared DPPH solution was added in duplicate. The mixture was incubated for 30mins and the absorbance was taken at 517nm using "micro plate reader".

$$Scavenging\ activity\ (\%) = \frac{[Abs\ S - Abs\ B]}{Abs\ C} x100$$

Where Abs S is the absorbance of sample, Abs B absorbance of blank and Abs C is absorbance of control.

2.4 Total phenolic content assay

2.4.1 Preparation of gallic acid standard solution

A total of 10 mg of pure gallic acid powder was accurately weighed and dissolved in 1 mL of methanol to prepare the stock solution. From this stock, working concentrations ranging from 0.2 to 0.005 mg/mL were obtained by serial dilution. For the test samples, 1 mg of each extract was dissolved in 1 mL of methanol to obtain the desired concentrations.

The prepared gallic acid standards were dispensed into a 96-well plate. Subsequently, 25 µL of each extract or standard solution was mixed with 125 µL of Lowry C reagent, followed by the addition of 75 µL of distilled water and 15 µL of Folin–Ciocalteu reagent. The mixtures were incubated for 40 minutes at room temperature, and absorbance was measured at 750 nm using a spectrophotometer.

2.4.2 Preparation of lowry reagents

- **Lowry A Solution**: 0.4 g of sodium hydroxide was dissolved in a 100 mL volumetric flask with distilled water up to the mark. Separately, 2 g of sodium carbonate (Na_2CO_3) was dissolved in 100 mL of 0.1 M NaOH solution.
- **Lowry B Solution**: 1 g of sodium potassium tartrate ($NaKC_4H_4O_6$) was dissolved in 100 mL distilled water to prepare a 1% solution. In another 100 mL volumetric flask, 0.5 g of copper sulfate ($CuSO_4$) was dissolved in the 1% sodium potassium tartrate solution.
- **Lowry C Solution**: Prepared by mixing 50 mL of Lowry A solution with 1 mL of Lowry B solution.

2.5 HPLC Analysis

Approximately 0.01 g of the sample was weighed and dissolved in 1 mL of methanol. The resulting solution was filtered and subjected to HPLC analysis for compound profiling.

3. Results of extraction and fractionation

The extraction yielded 6.469 g of ethanol extract and 3.921 g of chloroform extract.

3.1. Percentage scavenging activity of the extracts and the standards for DPPH

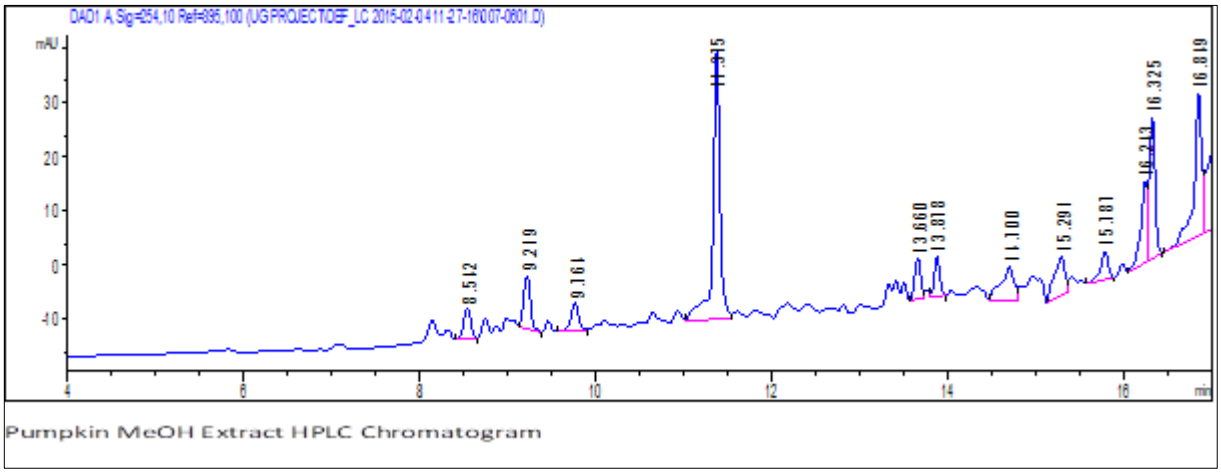
Table 1: Scavenging activity of various extracts and standards

Concentration (µg/ml)	Vit C %	BHT %	Crude ethanol Extract %	Methanol Extract %	Pet Ether Extract %	Black Tea %
1000	98.4169	99.1557	50.1279	45.2921	58.5225	89.1206
500	97.3028	96.8748	25.1724	36.6531	46.9459	78.1899
250	96.7986	96.37006	21.2481	24.1857	35.7897	73.7312
100	96.8221	89.6980	18.5138	19.9049	29.6724	63.3404
10	18.6632	46.0334	29.5330	16.8625	40.4541	39.5119

Antioxidant activity was observed after extraction, which was shown that vitamin c has higher antioxidant activity than that of the sample extract. Lower than pet ether extract.

The result of DPPH assay is expected to give the colour change and after extraction colour change from purple to yellow. The comparison between *Cucurbita pepo* samples and black tea shows that Black tea has higher activity then *Cucurbita pepo.* Comparison between vitamin C and samples shows that vitamin c is more active than *Cucurbita pepo* samples.

3.3. HPLC Chromatogram Showing Pe

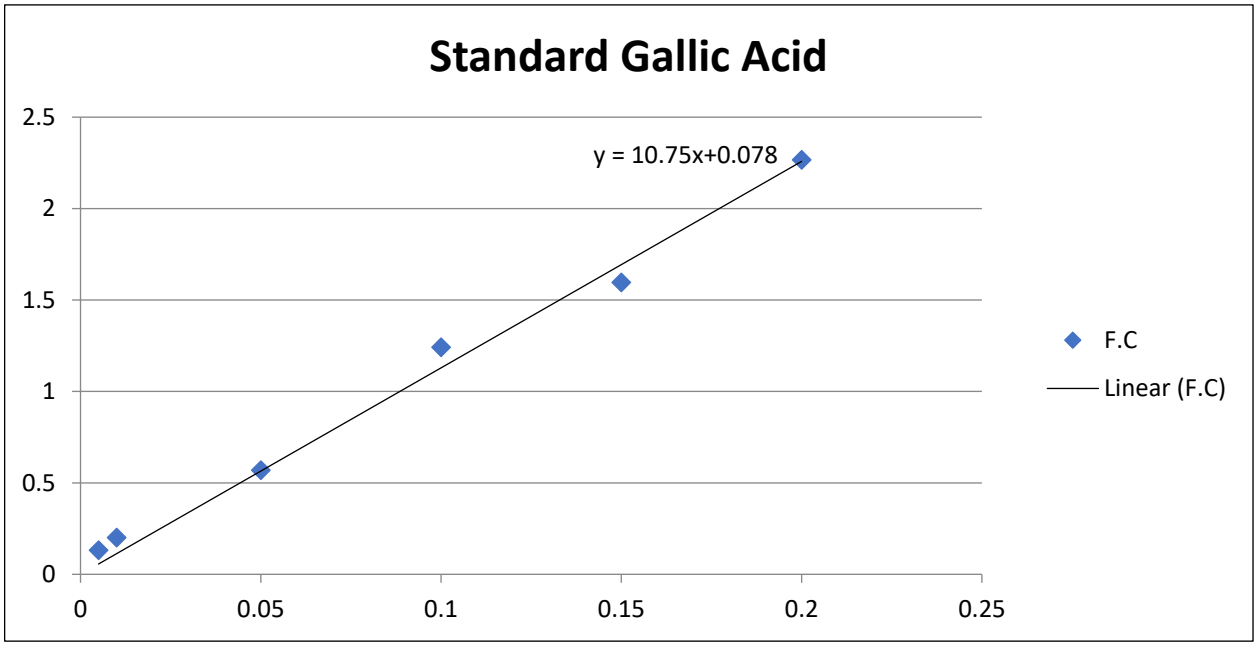


Pumpkin MeOH Extract HPLC Chromatogram

From the HPLC chromatograms, the results show the presence and different retention times of the compounds at 254 nm, demonstrating that the chromatograms closest to the origin are more polar than those farther away.

3.4. Total phenolic content

3.4.1. Standard calibration curve of gallic acid



3.4.2. Absorbance of Gallic Acid Equivalent to Extract

Crude extract	Absorption(mg/g) of gallic acid equivalent
Ethanol	8
Methanol	12.6
Pet Ether	17.1

Total Phenolic content indicated that the fraction of pet ether with 17.1mg/g of gallic acid equivalent has the highest number of phenols followed by methanol extract 12.6mg/g and ethanol fraction is the lowest with 8mg/g.

Conclusion

The results clearly indicate that *Cucurbita pepo* leaves serve as a natural source of antioxidants, as they exhibit significant antioxidant activity. When compared with other members of the Cucurbitaceae family, both the *C. pepo* sample and vitamin C demonstrated antioxidant potential; however, vitamin C exhibited higher activity than the *C. pepo* extract. High-Performance Liquid Chromatography (HPLC) analysis revealed the presence of multiple compounds eluting at different retention times. Furthermore, the total phenolic content of *Cucurbita pepo* samples was quantified using the Folin–Ciocalteu reagent.

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