Asian Journal of Synthetic & Natural Product Chemistry

Vol. 1, No. 1 (2023), 16-18
DOI: https://doi.org/10.14233/ajsnpc.2023.AJSNPC-P27003

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ARTICLE

Phytochemical Analysis and Antioxidant Activity of *Cucurbita pepo* L Leaves

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ARTICLE INFO

Article History:
Received: 29 November 2022
Accepted: 10 July 2023
Published: 7 November 2023

Keywords:
Cucurbita pepo L leaves, Antioxidant, Phytochemical, Food security, Nutritional challenges.

ABSTRACT

Cucurbita pepo L. leaves used as vegetables were randomly collected from the annual plant cultivated for its pumpkin in Harare, Zimbabwe to assess the presence of bioactive compounds in the leaves. Due to the presence of bioactive compounds, there will be a rise in the consumption of the leaves, which will help to bridge the nutritional gap and provide long-term food security. The leaves were shade dried in open air and ground into powder using mortar and pestle. The samples were separately soaked in distilled water and methanol, filtered and rotary evaporated to obtain the respective aqueous and methanolic concentrates. The concentrates were subjected to standard preliminary phytochemicals screening, assessment of free radical scavenging activity using α,α-diphenyl-β-picrylhydrazyl (DPPH) and GC-MS analysis. Preliminary phytochemical screening revealed the presence of flavonoids, steroids, saponins, tannins and alkaloids with the methanol extract being more efficient in the extraction of steroids. The extracts were nearly as effective as ascorbic acid in lowering DPPH free radical concentration. The GC-MS results revealed the presence of 7 phytoconstituents in methanol extract. Among these α-amyrin was detected and is considered to be medicinally important for new drug discovery.

INTRODUCTION

A number of consumable species of plants are considered insignificant, underutilized or ignored and have become part of the neglected and underutilized species (NUS) category of useful plant species [1]. Neglected and underutilized food crops exhibit great nutritional and healthy benefit values, however, their contribution in ensuring food security is poorly recognized and they are not included in countries diet and nutrition programs and policies [2]. Consumption of nutritive local foodstuffs will help to supplement the nutrients of the staple carbohydrates foods of the poor who cannot afford enough protein foods of animal origin. Knowledge of nutritive and medicinal benefits of local foodstuffs is necessary in order to encourage the increased cultivation and consumption to mitigate food and nutritional challenges.

Cucurbita pepo L. a member of the *Curcarbitacee* family widely grown in Southern Africa is one of the most overlooked and underutilized food and medicinal plant [3]. The pumpkins are used primarily in food and their seeds as herbal medicines. The *Cucurbita pepo* L leaves are palatable and are used as a vegetable. Its protein content of 21% is higher than those of other commonly used leafy vegetables [4]. The nutritional value of the *Cucurbita pepo* L leaves depends on the climatic

conditions where it is grown**.** In addition, *Cucurbita pepo* L leaves has health benefits such as antioxidant, antimicrobial, anti-inflammatory, cancer preventive, antidiabetic and antihypertensive effect [5].

In Zimbabwe, *Cucurbita pepo* L leaves are consumed as vegetables during the rainy season, however bioactive compounds of the Zimbabwean plant species are yet to be identified. Hence, the study was designed to determine the various phytochemicals and antioxidant activity in the *Cucurbita pepo* L. leaves, which evokes various therapeutic effect, in order to encourage increased cultivation and consumption.

EXPERIMENTAL

All the chemical reagents used in this research were of analytical grade. Desired solutions concentration ranges were prepared by serial dilution of the stock solution.

Plant material: The leaves of *Cucurbita pepo* L leaves were randomly collected from the local gardens in Harare Zimbabwe in the month of March 2023. The leaves were identified as *Cucurbita pepo* L. leaves (Muboora in Shona) by the botanist at Botanical Gardens Harare, Zimbabwe. The leaves were thoroughly washed with tap water to remove dust. The *Cucurbita pepo* L. leaves were then dried under shade at room temperature. The dried leaves were ground into powder using pestle and mortar. For subsequent use in the research, the powder was sealed in polythene container to avoid moisture contamination.

Preparation of extracts: Dried powder of *Cucurbita pepo* L. (200 g) were macerated in 100 mL methanol and 100 mL distilled water, respectively for 48 h to ensure complete the dissolution

Phytochemical screening: *Cucurbita pepo* L. leaf extracts were used for qualitative screening of the phytochemicals such as flavonoids, steroids, saponins, glycosides, tannins and alkaloids by standard biochemical procedures [6,7].

Antioxidant activity by DPPH assay: The DPPH free radical was evaluated as described by Gyamfi *et al*. [8]. The hydrogen atom or electron donating ability of the corresponding extract was measured from the bleaching of the purple coloured methanol solution of DPPH. This spectrophotometric assay uses the stable radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH), as a reagent. Ascorbic acid was used as standard in 0.2-1.0 mg/mL solution. DPPH (0.002%) was prepared in methanol. Briefly, appropriate dilution of the leaf extract was prepared by adding 9 mL of distilled water to 1 mL of concentrated sample. Each 0.6 mL of this mixture was dispensed into a test tube and 0.6 mL of DPPH solution was added and the mixture was left in the dark for 30 min, after that the absorbance was taken at 517 nm. DPPH solution (1 mL) was used as blank. The absorbance was recorded and inhibition was calculated as % scavenging activity using the following formula:

$$\text{Scavenging activity } (\%) = \frac{A_c - A_s}{A_c} \times 100$$

where A_c = control reaction absorbance; A_s = testing specimen absorbance [9,10].

GC-MS analysis: For the identification of bioactive compounds in the extracts, it was subjected to GC-MS analysis. It was performed using a JEOL GC MATE II instrument with the following conditions: Front inert temperature 220 ºC; column HP 5Ms; helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1mL/min. The oven temperature was 50 to 250 ºC@10 ºC/min. The ion chamber temperature and GC interface temperature was 250 ºC. The quadruple double focusing analyzer was used for mass analysis and the photon multiplier tube was used for detection. Mass spectra were taken at 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range 50-600 amu [11]. The composition of the crude extract constituents was expressed as a percentage by peak area.

Identification of compounds: The bioactive compounds in the methanol extract of *Cucurbita pepo* L. leaves were identified based on the GC retention time. Interpretation of mass spectrum of GC-MS was done by comparing with the data base at National Institute Standard and Technology (NIST). The name of the compound, molecular weight, molecular formula and structure of the compounds of the plant extracts were also retrieved from NIST, Guidechem, Chemspider and Pubchem Libraries [12].

RESULTS AND DISCUSSION

Preliminary phytochemical screening: The qualitative phytochemical screening results of secondary metabolites showed the presence of flavonoids, steroids, saponins, tannins and alkaloids as shown in Table-1.

TABLE-1
PHYTOCHEMICAL SCREENING RESULTS
FOR *Cucurbita pepo* L LEAVES

Phytochemical	Ethanol extract	Water extract
Flavonoids	Present	Present
Steroids	Present	Absent
Saponins	Present	Present
Glycosides	Absent	Absent
Tannins	Present	Present
Alkaloids	Present	Present

Antioxidant activity: The free radical scavenging activity of the two extracts methanol and water are as shown in Fig. 1. The percentage decrease in absorbance of DPPH due to the presence antioxidants in the extracts is shown. Activity of the extract decreased with an increase in time. A steep gradient showed high abilities of the samples to quench the DPPH radicals. The methanol extract had slightly lower free radial savaging activity than the distilled water extract. Both the alcoholic and aqueous extract of the *Cucurbita pepo* L. leaves possess antioxidant potential, which is slightly lower than that of the reference standard ascorbic acid. The strong antioxidant activity of the extract is due to the presence of flavonoids.

GC-MS analysis: The GC-MS analysis showed seven peaks in the GC-MS chromatogram (Fig. 2 and Table-2), which were identified according to their retention time. The identified compounds are 4*H*-1benzopyran-4-one,7-hydroxy-2-[4-methoxyphenyl]-, 4*H*-1-benzopyran-4-one,3,5,7-trihydroxy-2-[4-hydroxyl-3-ethoxyphenyl], 4*H*-1-benzopyran-4-one, 2-[3-chloro-2-hydroxyphenyl]-5-hydroxy-3,7,8-trimethoxy-, α-amyrin,

TABLE-2
CHEMICAL COMPOSITION OF METHANOLIC EXTRACTS OF *Cucurbita pepo* L LEAVES

Compound name	Retention time (min)	m.w. (d)	m.f.	Peak area (%)
4*H*-1-Benzopyran-4-one, 7-hydroxy-2-[4-methoxyphenyl]-	17.22	268	$C_{16}H_{12}O_4$	12.97
4*H*-1-Benzopyran-4-one, 3,5,7-trihydroxy-2-[4-hydroxyl-3-methoxyphenyl]-	17.98	316	$C_{16}H_{12}O_7$	12.68
4*H*-1-Benzopyran-4-one, 2-[3-chloro-2-hydroxyphenyl]-5- hydroxy-3,7,8-trimethoxy-	19.67	–	–	14.97
α-Amyrin	24.95	427	–	13.96
Dodecanoic acid, 1,1'-biphenyl-4-ylcarbonylmethyl ester	23.17	395	–	12.70
4*H*-1-Benzopyran-4-one, 5,7-dihydroxy-2-[2-methoxyphenyl-	17.53	–	–	13.34
Flavone, 2',3,5,7-tetramethoxy-	19.02	342	–	19.38

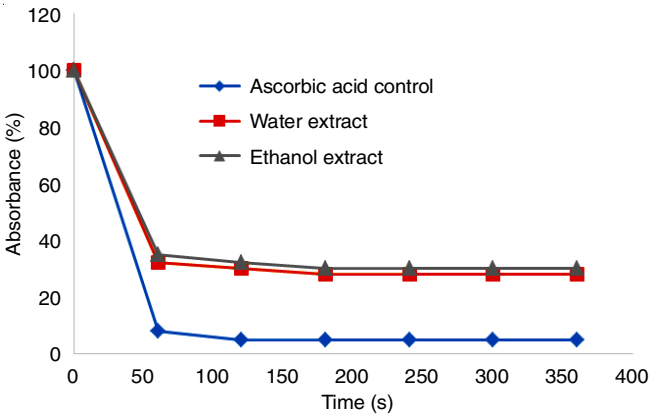

Fig. 1. Free radical scavenging activity of the two extracts

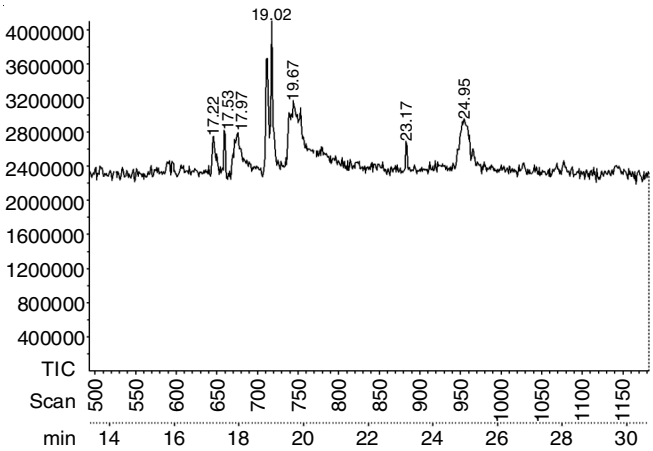

Fig. 2. GC-MS chromatogram of methanol extract of *Cucurbita pepo* L leaves

dodecanoic acid, 1,1-biphenyl-4-ylcarbonly methylester, 4*H*-1-benzopyran-4-one, 5,7-dihy-droxy-2-[2-methoxyphenyl]- and flavone, 2,3,5,7-tetramethoxy-.

Six groups of phytochemicals were screened in the methanol and aqueous leaf extract of *Cucurbita pepo* L leaves. The availability of a significant number of phytochemicals indicates that it has health benefits such as antioxidant, antimicrobial, anti-inflammatory, cancer preventive, antidiabetic and antihypertensive effect. The antioxidant activity results indicated that the leaves contain antioxidant compounds which effectively scavenged various free radicals and reactive oxygen species under *in vitro* conditions. The GC-MS results showed the presence of seven phytoconstituents in methanol extract which are medically important. Among them is α-amyrin which is medicinally important for new drug discovery.

The results showed that the leaves of *Cucurbita pepo* L. are a good source of natural exogenous antioxidants that are not carcinogenic and may be used to supplement the human body's natural antioxidants. The presence of bioactive compounds in *Cucurbita pepo* L. leaves will trigger increased cultivation and consumption to mitigate food and nutritional challenges and achieve the sustainable food security.

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