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Original Article

Free Radicals Scavenging Activity and Oxidative DNA Damage Protecting Property of Methanol Extract from *Honeycrisp* Apple

Williams Kweku Darkwah*, Matthew Nkoom

ABSTRACT

Objectives: This research reports the qualitative phytochemical constituents and considers the *in vitro* free radicals scavenging activities based on DPPH and nitric oxide assays and oxidative DNA damage protection activity of methanol extract from *Honeycrisp apple*. The foremost interest for this research was to use standard measures to determine nitric oxide scavenging activity, DPPH-scavenging activity and DNA damage protecting activity to assess the antioxidant potential of methanol extract from the apple. **Materials and Methods:** Concentrations of apple extracts with the intervals 20 µg/ml to 100 µmg/ml were prepared and mixed with suitable volumes of reagents and the corresponding absorbances read at the respective wavelength. **Results:** The outcomes of this research specify that methanol extract of *Honeycrisp apple* contain multiple phytochemical compounds that can expertly shield the body against oxidative stress caused by free radicals and might then be used as a source of potent natural antioxidant compounds. The IC_{50} values of methanol extract from *Honeycrisp apple* varied from 10.30 to 36.29 µg/ml which indicates the high radical scavenging activity of the sample. **Conclusion:** The DNA damage defensive potential of the extracts was also revealed, which could be used in cancer inhibition.

Key words: Honeycrisp apple, Free radicals, DNA damaging, Nitric oxide, Phytochemicals, DPPH.

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INTRODUCTION

Active cells in living organisms are always wide-opened to a range of challenges that work oxidative pressure in the system. Oxidative trauma rises in a living configuration once an augmented contact to oxidants, a decline happening the antioxidant ability of the system, or both. This is often associated with the production of reactive oxygen species (ROS), which consists of free radicals, which are intensely connected in the pathology of diseases, for instance cancer, rheumatoid arthritis, liver cirrhosis and arteriosclerosis also in degenerative processes associated with old age.[1-18]

Most Scientists doubted harmfulness of some artificial composites used in food and this upturned the interest in natural products.[11-31] Some productions, those in line with food additive production, cosmetics and pharmaceuticals, also have put up an augment on their struggles in the fabrication of bioactive composites mostly obtained from natural products synthesis. Free radical scavenging composites are well known to obstruct free radicals and upturn duration by delaying the course of lipid peroxidation.[16,17] Hence, this called for the need for isolating other forms of natural and nontoxic sources of food antioxidants.

Currently, research efforts have been dedicated to the studies on structure and physiological role of apple because of its recognized health remunerations.[3,5,13,22,28,32] The Scientific name of apple is *Malus domestica L. Borkh.* The health-protective possessions of apples such as honeycrisp are attributed to phytochemicals, such as phenols that may reduce the menace of obesity, diabetes, cardiovascular disease and cancer through protection against oxidative damage.[10,26,33,34]

The prime purpose of this research was to determine the qualitative phytochemical constituents and consider the *in vitro* free radicals inhibitory activities based on DPPH and nitric oxide assays and oxidative DNA damage protection activity of methanol extract from honeycrisp apple. This work will provide a basis for the use of honeycrisp apple as a functional food or natural medicinal ingredient that can replace synthetic compounds.

MATERIALS AND METHODS

Plant material

Ripe *Honeycrisp* apple fruits were obtained from 10-year-old apple trees implanted on the rootstock in the botanical garden, University of Cape Coast, Ghana in October 2015. The defect-free apples were collected. The taxonomic character of the apple was confirmed by a plant taxonomist at the Department of Botany, University of Cape Coast, Ghana. The apple sample was then washed under running tap water to eradicate undesirable filth and other external materials. The sample was look - dried underneath shade till the absence of moisture. The dried sample was grounded into powder by the help a blender.

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Preparation of plant extracts

Methanol extraction

The methanol extract was prepared by soaking 60 g of powdered sample of *Honeycrisp apple* in 210 ml of methanol (70%) for 72 h at room temperature (35°C). The mixture was then filtered using Whatman filter paper No 1. The filtrate was intense under a low pressure using rotary evaporator at temperature of 46°C. The follow-on extract was weighed and stored in airtight bottles at room temperature for further used.

$yield\ (\%) = \left(\frac{A_1}{A_0}\right) \times 100$, Where A_0 was the mass of the sample and A_1 was the mass of the crude extract.

Phytochemical screening (qualitative analysis)

Phytochemical selection of the methanolic extracts of *Honeycrisp apple* was carried out as per the standard protocol[6,7,18,33] to determine the presence of glycosides, terpenoids, flavonoids, carbohydrates, protein, alkaloids, phenolic compounds, tannins, saponins and Phytosterols.

1, 1 Diphenyl-2, picrylhydrazine (DPPH) antioxidant activity

This was measured in accordance with the method of Shimada, *et al.*[29] Each reaction mixture contained 1.0 mL of a specific concentration (20-100 µg/mL) of methanol extract of *Honeycrisp apple* and standards (ascorbic acid and tannic acid) and 1.0 mL of DPPH solution (0.135 mM). The mixture was shaken vigorously and left in the dark for 30 mins. The absorbance was measured at 517 nm against a reagent blank. The percentage ability for scavenging DPPH free radical by the sample was designed in accordance with to the equation:

$DPPH\ scavenging\ activity\ (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$, Where A_0 was the blank absorbance and A_1 was the extract absorbance or the standard absorbance

Nitric oxide scavenging activity

Nitric oxide free radical inhibition was measured in accordance with the technique designated by Jagetia, *et al.*[19] A volume of 2 ml sodium nitroprusside in phosphate buffer (0.02 M, pH 7.4) was varied with diverse concentrations (20- 100 µg/ml) of each methanol extract of *Honeycrisp apple* and standards (ascorbic acid and tannic acid). The feedback combination was retained at 25°C for 2 hrs. Subsequently, 1.5 ml of Griess reagent [1% sulphanilamide, 2% O-phosphoric acid and 0.1% of N-(1- naphthyl) ethylenediamine dihydrochloride] was added. The absorbance measurement was taken at 540 nm 30 mins afterwards against a phosphate buffer blank. The percentage inhibition of nitric oxide radicals by extracts and standards was extrapolated using the formula:

$scavenging\ activity\ (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$, Where A_0 was the blank absorbance and A_1 was the extract absorbance or the standard absorbance.

Genomic DNA extraction

Cowpea seeds (*Vigna unguiculata*) purchased from Kotokuraba market, Cape Coast, in the Central region of Ghana, were sowed and allowed to germinate. After 1 week, the leaves of the germinated seeds were collected. Genomic DNA was extracted adopting a modified cetyltrimethylammonium bromide protocol described by Darkwah, *et al.*[7,36] The integrity of the extracted DNA was tested by running a 5 µl DNA sample on gel electrophoresis at 100 V for 1 h 20 mins. The purity was also determined by measuring the absorbance of each DNA sample at 260 nm and 280 nm and the ratio computed. A ratio ≥ 1.8 ≤ 2.0 implied a pure nucleic acid sample; however, a ratio < 1.8 indicates impurity depicting the presence of protein. Meanwhile, a ratio > 2.0 indicates that the samples were contaminated with phenol or chloroform. To standardize the samples, the concentration of the DNA was also determined using the formula:

$$\text{DNA concentration } (\mu g / ml) = \left(\frac{A_{260} * D * 50\mu g/ml}{1000}\right)$$

Where A_{260} represents the absorbance at 260 nm and D is the dilution factor. The determination of the concentrations enabled the standardization to 100 ng/µl. Samples that were highly concentrated were diluted with sterile Tris−EDTA (TE) buffer, whereas those with lower concentrations, the extraction process was repeated.

DNA damage protective potential of methanolic of Honeycrisp apple

DNA protection potential of the extracts were evaluated using cowpea (*Vigna unguiculata*) genomic DNA. DNA was extracted according to the cetyl trimethyl-ammonium bromide (CTAB) technique as designated by Doyle and Doyle.[7,9] Oxidative impairment to DNA was prompted using hydroxyl free-radical generating system (H_2O_2/UV) which was also pronounced by Russo, *et al.*[27] in the existence of the extracts. Briefly, 10 µl aliquot of cowpea DNA was added into microfuge tubes containing 10 µl of different concentrations of methanol extracts (0.02, 0.1 and 10 mg/ml) and 10 µl of 30% H_2O_2. Ascorbic acid (10 mg/ml) was used as a constructive control in the absence of the *Honeycrip* apple sample. The normal control contained only the DNA extract while the destructive control contained DNA plus H_2O_2. The tubes were UV irradiated using UV transilluminator (UVP Upland, CA 91786 U.S.A.) for 1 hr 20 mins at regular room temperature. After treatment, 5 µl of 6X bromophenol blue was added to each tube. All samples were examined by gel electrophoresis on 1% agarose gel (containing ethidium bromide) in TAE buffer (pH 8). The gel was then photographed under UV light. Untreated, cowpea DNA was lane along with untreated UV-irradiated DNA and extracts treated UV-irradiated sample.

Statistical analysis

All tests were conducted in triplicate. Data are reported as means ± standard error (SE). Results were analyzed statically by using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Phytochemical analysis of methanol extract of Honeycrisp apple

The phytochemical exploration validated the manifestation of alkaloids, flavonoids, glycosides, phenols, saponins, quinones, carboxylic acids, proteins and tannins (Table 1). Phenolic compounds, flavonoids, alkaloids and tannins are deliberated to hold great antioxidant

Table 1: Shows phytochemical constituents of methanol extract of *Honeycrisp* apple.

Test	Methanol extract
Alkaloid	++
Proteins	+++
Tannins	++
Saponnins	+
Glycosides	+++
Quinones	+
Flavonoids	+++
Carboxylic acid	++
Phenols	+++

Present (+), absent (-).

potentials, which avert or can be used in the management of many diseases, comprising cancer.[6,23,35] As a result, the existence of significant to realistic amounts of these phytochemicals can be linked with the promising significant antioxidant prospective of the apple.[6,12,25]

Table 1 displays phytochemical constituents of methanol extract of *Honeycrisp* apple. It was detected that all photochemical under investigation confirmed positive.

Antioxidant activity of methanol extract from Honeycrisp apple

The harmfulness and destruction caused by NO^- with superoxide anion is increased as they retort to yield reactive peroxynitrite (ONOO), which hints to solemn lethal feedbacks with biomolecules such as protein, lipids and nucleic acids.[5,30] In this research, the despicable inhibition was improved with cumulative concentration of the extract and the standard ascorbic acid was moderately more noticeable than that of the extract. The standard ascorbic acid and the extract has the highest % inhibition of 93.98% and 61.50% respectively for the highest concentration tested (100 µg/ml) (Figure 1). Reactive oxygen species, NO is also occupied in inflammation, cancer and other biological situations.[6,24] The NO^- scavenging activity of *Honeycrisp apple* may assist to capture the chain reactions introduced by additional cohort of $ONOO^-$ that is disadvantageous to human condition.

This illustrates that methanol extract had the ability to rummage nitric oxide radicals. The % inhibition increases with growing concentration in a measured quantity dependent way (Figure 1).

Additionally, the extract also revealed DPPH sifting action. DPPH is an unwavering free radical and has been useful widely in the assessment of the capacity of natural products to turn as free radical scavengers or hydrogen donors.[21] Antioxidants whichever relocate an electron or a hydrogen atom to DPPH, thus cancelling out a number of DPPH molecules identical to that of hydroxyl groups.[2] The standard ascorbic acid, tannic acid and the methanol extract has the highest % inhibition of 56.68% for the highest concentration tested (100 µg/ml), 44.06% for 80 µg/ml concentration tested and 40.92% for the highest concentration tested (100 µg/ml) respectively (Figure 2). This seems that *Honeycrisp* apple retains hydrogen-donating abilities to perform as an antioxidant. Centered on the figures achieved from this study, the scavenging abilities improved with collective concentration of the extract. The results give a picture that the extract had the very aptitude to scavenge DPPH - radicals. The % inhibition increases with cumulative concentration in a measure dependent mode (Figure 2).

The IC_{50} is inversely proportional to the scavenging activity of the extract. The IC_{50} values of methanol extract from *Honeycrisp apple* varied from 10.30 to 36.29 µg/ml (Table 2) which indicates the high radical scavenging activity of sample. The IC_{50} were in the order: Ascorbic acid > Tannic acid > *Honeycrisp apple* and Ascorbic acid > Honeycrisp apple for DPPH % inhibition activity and NO^- % inhibition activity respectively (Table 2).

Oxidative DNA damage protecting activity of methanol extract from Honeycrisp apple

The UV photolysis of H_2O_2 involves the reaction between H_2O_2 and UV to form hydroxyl radical. The results showed complete degradation of genomic DNA from cowpea treated with UV indicated in lane 2 (Figure 3). The action of the apple extract on DNA damage was indicated in lanes 3 and 4, 7 and 8 for the extract and DNA alone and 5 and 6 for the

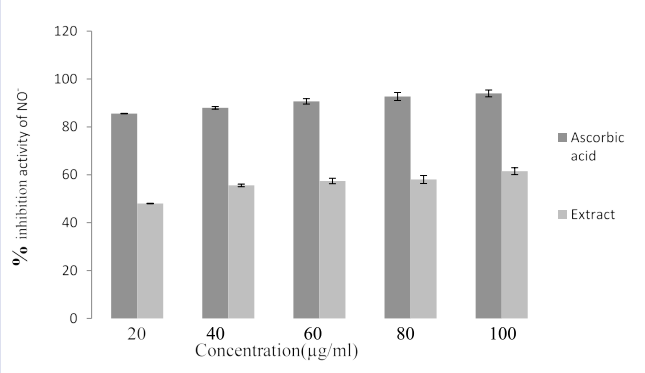

Figure 1: Displays the nitric oxide inhibition of the methanol extract of *Honeycrisp* apple and standard, ascorbic acid.

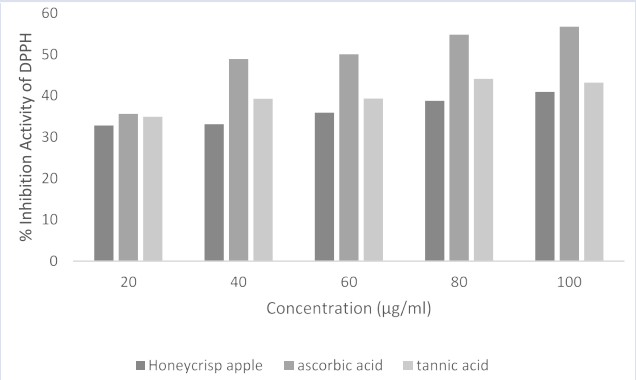

Figure 2: Displays DPPH - scavenging activity of methanol extract from *Honeycrisp apple* paralleled per standard ascorbic acid and tannic acids.

Table 2: IC_{50} values of free radical scavenging activities of methanol extract from *Honeycrisp* apple (µg/ml).

Sample	DPPH	NO
Honeycrisp apple	36.29±1.67	10.30 ± 0.30
Ascorbic Acid	49.18±1.86	12.20 ± 0 .32
Tannic Acid	40.14±0.7	------

Values represent the mean of three readings ± standard deviation of the mean.

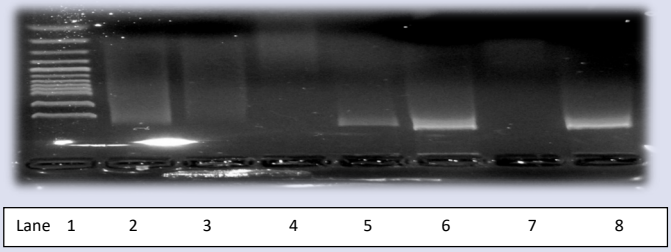

Figure 3: Expresses the electrophoretic arrangement of DNA from cowpea after UV-photolysis of hydrogen peroxides in the presence and absence *Honeycrisp apple* extract. Lane 1 : (marker), lane 2 : (DNA + H_2O_2), lane 3 : (DNA + H_2O_2 + 20 µg/ml extract), lane 4 : (DNA + H_2O_2 + 100 µg/ml extract), lane 5 : (DNA + H_2O_2 + 20 µg/ml ascorbic acid), lane 6 : (DNA + H_2O_2 + 100 µg/ml ascorbic acid), lane 7 : (DNA + 100 µg/ml extract), lane 8 : (DNA + 20 µg/ml).

standard ascorbic acid (Figure 3). From figure 3, it could be concluded that the extract and ascorbic acid showed some DNA protection ability, but the effect is not much seen when the concentrations were varied. The strength of the DNA damage was condensed on a concentration dependent manner of the extracts towards DNA which shows the shielding influence of the extract towards hydrogen peroxide made damage. The result suggests that methanol extract of *Honeycrisp apple* protect DNA through antioxidant activity and may be used in future to prevent cancer.[7,20] The result agrees with other reports which indicate the potential of plants to protect against free radical-mediated DNA damage.[7,14,27]

CONCLUSION

In conclusion, this research exposed the presence of polyphenolics and some other phytochemicals in the methanol extract from *Honeycrisp apple* which also reflected in the free radical scavenging activity of this extract. Further, the extract conferred fortification against oxidative destruction. The antioxidant activity of *Honeycrisp apple* may possibly substantiate advance enquiry of its other constructive natural possessions and determine its protection.

ACKNOWLEDGEMENT

Not applicable.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

ONOO-: Reactive peroxynitrite; NO: Nitric oxide; UV light: Ultraviolet light; DPPH: 1,1-Diphenyl-2, picrylhydrazine; ROS: Reactive oxygen species.

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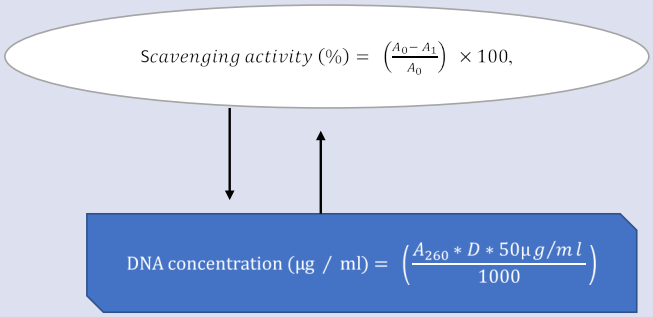
GRAPHICAL ABSTRACT



SUMMARY

- This report presents the qualitative phytochemical constituents and considers the *in vitro* free radicals scavenging activities of methanol extract from *Honeycrisp apple.*
- It tackles the activities based on nitric oxide assays and related, and oxidative DNA damage protection activity
- The DNA damage defensive potential of the extracts was also revealed, which could be used in cancer inhibition.

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