International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 10 Issue: 12 | Dec 2023 www.irjet.net p-ISSN: 2395-0072

EXTRACTION OF ANTIOXIDANTS FROM ONION PEEL

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Abstract *In the food sector, onion peel is considered waste since improper disposal might contaminate the environment. However, this waste from onion peels can be used as a renewable raw material for the extraction of antioxidants because it is rich in various molecular species of antioxidant chemicals. In order to extract antioxidants from this source and study their antioxidant properties, an environmentally friendly extraction process has been created. Proximate analysis was performed on red onion peel (Allium cepa), and the extract's total phenol, total flavonoid, and total anthocyanin contents as well as its capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals were investigated. The information reported in this study showed that the extract made from onion peels produced*

Key Words: **Extraction, Antioxidant, Onion Peel**

1.INTRODUCTION

Onions (Allium cepa) possess strong characteristic aromas and flavors, which have made them important ingredients in food. It has been shown that bioactive compound present in every part of onion bulb. Plants have been used to treat chronic as well as infectious diseases since antiquity. The biological activities of plants have been attributed to the presence of various secondary metabolites such as alkaloids, glycosides, phenols, flavonoids, coumarins, volatile oils etc. Phenolic compounds are known for their antioxidant properties and play a vital role in the Onion is a potent cardiovascular and anticancer agent, with hypocholesterolemia, antioxidant, anti- asthmatic, and antithrombotic activity. Onion is of the major sources of dietary flavonoids which contains anthocyanins, that is Introduction prevention and management of many chronic diseases such as cancer, diabetes, cardiovascular and neurodegenerative diseases. Currently natural antioxidants are gaining popularity due the belief that they are safer and provide more health benefits than synthetic antioxidant which have numerous health hazards. Thus, plants containing phenolic compounds are potential reservoir for discovery of effective and safe antioxidants. for the red or purple color observed in some varieties, and flavanols (quercetin) that may contribute to the production of yellow and brown compounds found in the skins of many onions. Quercetin has demonstrated antioxidant and free radical scavenging power and its capability to protect against cardiovascular disease. However, onion skins contain higher concentrations of quercetin aglycon than the flesh. It has been reported that quercetin and quercetin 4'-O-glucopyranoside are the major flavonoids in red onion peel. Quercetin, a bioflavonoid extracted from red onion peel, showed marked inhibitory activity against phosphodiesterase 5A.

Extraction is a first important step to recover the bioactive compounds from natural sources. The choice of solvent for extraction of phenols is a critical step as solubility of different classes of phenols is not similar in different solvents. Therefore, several solvent systems have been used for the extraction of plant phenolics and antioxidant compounds. Generally, polar solvents like aqueous mixtures of acetone, ethanol and methanol are employed for the extraction of plant phenolics Thus, the present work was designed to evaluate the effect of different polar solvents on extraction of valuable antioxidant phytoconstituents from onion waste.

1.1 Methodology

1.1.1 Instruments and Equipment's

Soxhlet Extractor, Tray dryer, Spectrophotometer, Rotary evaporator, Condenser2800-micron Sieve, Filter paper, Measuring Cylinder, Conical flasks

1.1.2 Chemicals

Ethanol,2 N Hydrochloric acid, Distilled water, FeCl3, AlCl3, Folin-Ciocalteu's Phenol Reagent, Sodium hydroxide, Sulphuric Acid, Ammonia, Sodium Carbonate

1.1.3 Raw material

Onion peels were purchased from local market.

1.1.4 Solvent Selection Criteria:

Polarity and molecular affinity, Boiling point, Viscosity, Availability and Cost, Toxicity, Volatility, Good solubility of targeted compound

1.1.5 Process flow diagram

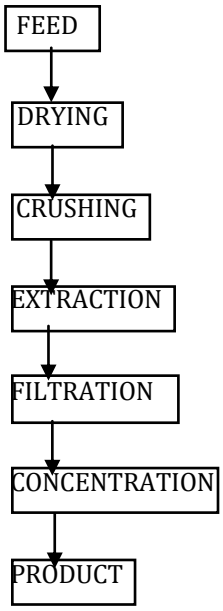

Fig.1.1: Block diagram Extraction of antioxidant from onion peel

1.1.6 Preparation of sample

a) The peels were washed with tap water and cleaned from impurities.
b) The peels were dried in the tray dryer at 105 ℃ for 5 hrs.
c) The peels were then crushed into powder and passed through 2800-micron sieve. The sizes being reduced in order to increase the surface area for uniform mixing with chosen solvent.
d) The sample powder will then be vacuum packed and stored in freezer until used for analysis.

1.1.7 Experimental setup

Assembly-

a) The source material containing the compound to be extracted is placed inside the thimble.
b) The thimble is loaded into the main chamber of the Soxhlet extractor.
c) The extraction solvent to be used is placed in a distillation flask.
d) The flask is placed on the heating element.
e) The Soxhlet extractor is placed atop the flask.
f) A reflux condenser is placed atop the extractor.

Operation-

a) A reflux is reached by heating the solvent.
b) The vapor from the solvent ascends a distillation arm and fills the chamber that holds the solid thimble.
c) The condenser makes sure that any solvent vapor cools and returns to the solid material-containing chamber by dripping back down.
d) The warm solvent gradually fills the chamber that holds the solid substance. In the heated solvent, some of the desired chemical dissolves.
e) The siphon empties the Soxhlet chamber when it is nearly full. The distillation flask is filled with the solvent once more.
f) The thimble makes sure that no solid material is transferred to the still pot by the solvent's fast motion.
g) This cycle could be continued for several hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. For removal of solute particles from the extract use filter paper. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

Fig 1.1 Experimental Setup Soxhlet Extractor

Fig 1.2 Extraction

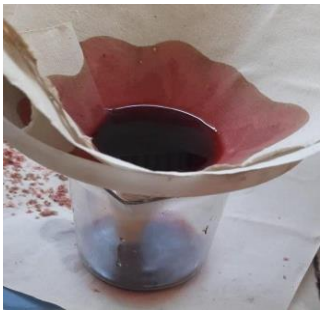

Fig 1.3 Filtration

Fig 1.4 Extract

Table 1.1 Operating condition

Solute-to-solvent ratio	1:10 [5]
Solute	20 g
Solvent	200 ml (140 ml Ethanol + 60 ml HCl)
Temperature	79.77 ℃ (Mixture B.P.)
Pressure	1 atm

2.Confirmation test:

2.1Flavonoid confirmatory test: -

In 1 ml extract add few drops NaOH which gives greenish yellow color. To this add few drops of 2N HCl, yellow color disappears.

2.2Anthocyanin confirmatory test: -

In 1 ml of extract add 1 ml of HCl which gives Pinkish-red color. In 1 ml of extract add 1 ml of NaOH which gives Greenish yellow color.

2.3 Rotary Evaporator: -

Extract is collected and concentrated with a rotary vacuum evaporator.

Table 1.2- Operating conditions of rotary vacuum evaporator

Temperature	40° C
RPM	100
Time	1 hr.

3 Analysis

3.1 Determination of total phenol: -

Method – Folin - ciocalteu's method.

Procedure-

a) 1 ml of extract is mixed with 10 ml of deionized water and 1.5 ml Folin-Ciocalteu's reagent.
b) After 5 min, 4 ml Na2CO3 (20%, w/v) is added and volume is adjusted to 25 ml with deionized water.
c) After 30 min of incubation, the absorbance is measured using spectrophotometer at 760 nm.

Calculations-

a) Gallic acid is used as reference standard.
b) The result is expressed as milligram gallic acid equivalents (mg GAE)/g of extract.

3.2 Determination of total anthocyanin: -

Method - pH differential method.

Procedure-

A spectrophotometer is used for the spectral measurements at 210 nm and 750 nm.

Calculations-

Absorbance of the samples (A) = (Absorbance λ vis-max-A750) pH 1.0 - (Absorbance λ vis-max-A750) pH 4.5
Anthocyanin content = (A*MW*DF*1000) / (ε*1). (mg/lt) were,
M.W. of anthocyanin (cyd-3-glu) = 449 Extraction coefficient (ε) =29,600 DF=Diluted factor

3.3Determination of total flavonoid content: -

Method - Aluminum chloride colorimetric method.

Procedure -

a) Extract (0.1g) is dissolved in 1 ml deionized water.
b) Dissolved sample solution (0.5 ml) is separately mixed with 95% Methanol (1.5 ml), 10% Aluminum Chloride (0.1 ml), 1 M Potassium acetate (0.1 ml) and deionized water (2.8 ml).
c) After 40 min, the absorbance of resultant mixture is measured spectrophotometrically at 415 nm against a deionized water blank.

Calculations-

a) Quercetin is used as reference standard.
b) Result is expressed as milligram quercetin equivalents (mg QE)/ g of extract.

3.4Determination of Antioxidant activity -

Procedure

Scavenging ability against DPPH: - The free radical scavenging ability of the extract is evaluated against DPPH (1,1-diphenyl-2- picrylhydrazyl) free radical I order to determine its antioxidant activity.

1 ml of the extract is mixed with 1 ml of 0.1 mM DPPH methanolic solution.

The mixture is then incubated for 30 min at 25 C, in dark. iv.

The absorbance is measured at 515-517 nm.

Calculations-

The inhibition of DPPH radicals by the sample is calculated according to the following equation:

DPPH-scavenging activity (%) = [1- (absorbance of the sample / absorbance of the control)] x 100

Were,

Control – Methanol

4.Results: -

Table 1.3: Parameters and results of Extraction and Concentration

For Extraction	For rotary evaporator
Operating temperature - 79°C	Operating temperature - 40°C
Time – 2hr	Time - 1 hr.
Solvent - 200 ml	RPM - 100
Raw material - 20 g	Solvent recovery - 50 ml
Extract - 125 ml	Extract - 75 ml

Table 1.4: Total content of antioxidants in the extract

Assays	Total content
Total anthocyanins content	62.19 (mg/g)
Total flavonoid content	418.0 + 34.4 (mg GAE/g of extract)
Total Phenolic content	212.3 + 14.6 (mg QE/g of extract)
DPPH	43.7 (IC50 microgram/ mg)

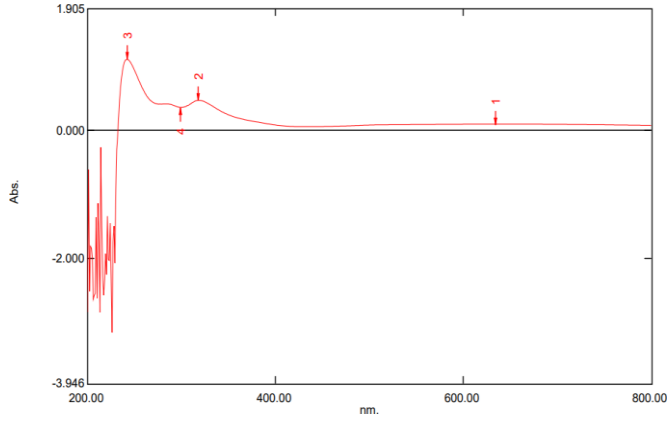

Fig.1.5: Graph of spectrophotometric analysis for Phenolic compounds

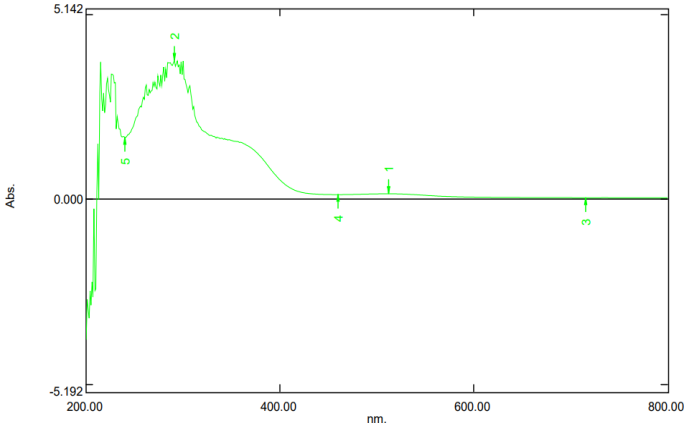

Fig.1.6 Graph of spectrophotometric analysis for Anthocyanins

5.Conclusion: -

The food industry produces a large amount of onion waste and there is need to search for possible ways of its utilization. In this study, we have found that onion peel has high content of anthocyanins, flavonoid, and phenolic compounds. Also, antioxidant activity of onion peel is well known and reported in this literature. Therefore, it may be suggested that after proper cleaning and processing, onion peel may be utilized as a source of natural antioxidants in food processing instead of discarding it. Also, further studies should be done to investigate the capability of onion peel to serve as a functional ingredient in food formulations.

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