Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(1): 502-505
Received: 16-11-2016
Accepted: 17-12-2016

Varinder Singh
Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, Punjab, India

Pawan Krishan
Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, Punjab, India

Richa Shri
Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, Punjab, India

Correspondence
Richa Shri
Professor, Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, Punjab, India

Extraction of antioxidant phytoconstituents from onion waste

Varinder Singh, Pawan Krishan and Richa Shri

Abstract
Allium cepa L. is valued in traditional as well as modern systems of medicine. It is utilized as a major vegetable crop worldwide. However its use is plagued by generation of large quantities of outer scales discarded as waste. Literature shows that this leftover is rich in phenolic compounds. The present study aimed at establishing the most appropriate solvent for recovery of the antioxidant phenolic compounds. Different polar extracts were prepared and their total phenol content (TPC), total flavonoid content (TFC) and antioxidant activity were used as quality indicators to evaluate the extraction efficiency of the solvents. Distilled water extractive value was the highest (16.76 % w/w); while 70% methanol extract had the maximum TPC, TFC and DPPH scavenging activity (IC_{50} of 30.1µg/ml). Thus, methanol: water (70:30) may be used at laboratory scale as well as commercially to recover valuable phenolic compounds and flavonoids from onion waste.

Keywords: Antioxidant, Extraction solvent, Flavonoids, Onion outer scales, Phenols

Introduction
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 [1, 2]. Phenolic compounds are known for their antioxidant properties and play a vital role in the prevention and management of many chronic diseases such as cancer, diabetes, cardiovascular and neurodegenerative diseases [3, 4]. 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 [5, 6]. Thus plants containing phenolic compounds are potential reservoir for discovery of effective and safe antioxidants.
Allium cepa (Liliaceae), commonly known as onion and commercially cultivated worldwide, is one of the richest sources of phenols and flavonoids [7]. Onion bulbs are traditionally used to treat various diseases like asthma, bacterial infections, bronchitis, common cold, ulcer wounds, cholera, colic, earache, fever, hypertension, jaundice, pimples and sores [8-10]. It possesses a multitude of biological activities including anti-carcinogenic, anti-hypertensive, anti-microbial, anti-spasmodic, anti-thrombotic and neuro protective [10-13].
Onions are important commercially as well as medicinally. However, generation of large quantities of waste (for example 450,000 tons of onion waste is generated annually in European Union) from onions necessitates its proper management [14]. Since onion waste has strong aroma and provides the media for growth of phytopathogens, it cannot be used for fodder and landfill disposal [15]. Moreover due to large moisture content its disposal by combustion is expensive [14]. The skin or outer scales of onion bulbs (i.e. onion waste) is reported to contain high content of phenolics and flavonoids, among them quercetin dominates [16, 17]. Thus there is a need to manage onion waste in such a way that the undesirable leftover plant material is eliminated and may be used for production/isolation of some useful phytochemicals that may otherwise be lost with the waste.
By-products and wastes of plant food processing can be used to retrieve important bioactive compounds. Numerous studies have been done with the aim of development of efficient processes for recovering bioactive phytochemicals [6, 18-20].
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 [21]. 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 [22].

Thus, the present work was designed to evaluate the effect of different polar solvents on extraction of valuable antioxidant phytoconstituents from onion waste.

Materials and Methods

Chemicals

DPPH (2, 2-diphenyl-1-picrylhydrazyl) was procured form Sigma-Aldrich Co., St. Louis, MO, USA. Ascorbic acid, Folin Ciocalteu's reagent, sodium carbonate and methanol were used as supplied by Loba Chemie, India. Gallic acid was procured from CDH chemicals, India. All other chemicals used were of analytical grade and used as received.

Plant material

The bulbs of *Allium cepa* var. NHRDF RED were collected from its cultivated source National Horticulture Research and Development Foundation (NHRDF), Bathinda, Punjab, India in July, 2013. The variety is released by NHRDF and notified by Government of India. The bulbs were authenticated by Sh. H.K. Sharma, Assistant Director, NHRDF, Bathinda.

Extraction of plant material

The outer scales of onion bulbs were separated, made free from soil, shade dried and coarsely powdered. The powdered scales (40 g) were extracted separately with 150 ml each of distilled water, methanol, 70% methanol and 70% ethanol by ultrasonication on bath sonicator for 30 min followed by maceration (24 hrs) in shaking incubator at 37±0.2^0 C. The extracts obtained were concentrated under vacuum and percentage yield with to respect to dried weight of plant material was calculated.

Determination of total phenolic content

Total phenolic content of all extracts was determined according to the method reported by Singleton *et al*. with slight modifications [23]. In brief, one ml of all dissolved extracts was mixed, separately, with 10 ml of deionized water and 1.5 ml undiluted Folin Ciocalteu's reagent. After 5 min, 4 ml Na_2CO_3 (20%, w/v) was added and volume was adjusted to 25 ml with deionized water. The mixture was incubated for 30 min and absorbance of resulting mixture was measured spectrophotometrically (Beckman DU 640B, Nyon, Switzerland) at 765 nm. The assay was performed in triplicate. Gallic acid was used as reference standard and the results were expressed as milligram gallic acid equivalents (mg GAE)/g of extract.

Determination of total flavonoid content

The total flavonoid content was determined by aluminum chloride colorimetric method [24]. Briefly, all extracts were, respectively, dissolved in 80% methanol. Each dissolved sample solutions (0.5 ml) was 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). After 30 min, the absorbance of resultant mixture was measured spectrophotometrically (Beckman DU 640B, Nyon, Switzerland) at 415 nm. The assay was performed in triplicate. Quercetin was used as reference standard and the results were milligram quercetin equivalents (mg QE)/g of extract.

Antioxidant activity of extracts

Determination of the free radical scavenging activity of the different extracts was carried out using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. DPPH radical scavenging assay of different extracts was performed by method reported by Blois, with slight modifications [25]. Briefly, equal volume of methanolic solution of DPPH (0.1 mmol/l) and extracts at different concentrations (1- 200 µg/ml) were mixed with vortex shaker and incubated (30 min at 30 ^{0}C). Ascorbic acid was used as positive standard control. The assay was performed in triplicate. The absorbance of the resulting solution was read at 517 nm against a blank. The DPPH radical scavenging effect was measured as

$$\% \text{ scavenging effect} = \left(1 - \frac{a}{b}\right)$$

Where, a= Absorption of sample solution at 517 nm
b= Absorption of control solution at 517 nm
The concentration of extract required to scavenge DPPH free radical by 50% (IC_{50}) was estimated from the curve of scavenging effect (%) plotted against the respective concentrations by using linear regression.

Results and Discussion

Effect of solvents on extraction

Choice of solvent plays a vital role in the extraction of phytochemicals. Earlier studies report the use of polar solvents for effective extraction of phenolic compounds [36]. Investigations on onions have also shown that methanol and ethanol are effective for isolation of phenols and flavonoids [37, 38]. To study the effect of different solvents on extractive value, total phenolic content and total flavonoid content four different solvents viz. methanol, distilled water, 70% methanol and 70% ethanol were selected. The percentage yields of extracts obtained with different solvents is reported in Table 1. Distilled water gave maximum yield for onion skin extract while methanol gave the lowest value. The extraction yield increase in order: methanol < 70% methanol < 70% ethanol < water.

Table 1: Yields of extracts of *Allium cepa* var. NFRDF-Red prepared with different solvents

Solvent used	% yield (w/w)
Methanol	5.53
Distilled Water	16.76
70 % Methanol	5.80
70 % Ethanol	5.92

Effect of solvents on total phenol and flavonoid content

The total phenol and flavonoid contents of prepared extracts were estimated using the Folin-Ciocalteu and aluminum chloride method respectively, which are simple and widely used methods [26-28]. Extraction with 70% methanol yielded maximum phenol and flavonoid content (Table-2) while distilled water gave extract with lowest contents. Further, 70 % ethanol gave relatively lower phenol content than 70 % methanol.

Binary solvents are reported as better solvent system for extraction of phenolic compounds as compared to mono solvents [29, 30]. Moreover, Jayaprakasha *et al*. reported a low yield of plant phenolics when methanol and acetone were used alone as solvent [31]. Further, aqueous mixtures with methanol, ethanol or acetone were found better solvent for extraction of phenolic compounds, from *Vitis rotundifolia* seeds, than mono-component solvent [32]. It was revealed that hydro-methanol (85%) gave maximum yield of phenols and flavonoids from the apples [33]. The possible reason could be that some phenol compounds exist as glycosides and presence of sugar moiety makes them more water soluble [34].

Table 2: Total phenolic and flavonoid content in different extracts of onion waste

Solvent used for extraction	TPC (mg GAE/g of extract)	TFC (mg QE/g of extract)	DDPH Assay (IC_{50} µg/ml)
Methanol	389.5±4.9	178.7±25.0	83.7
Distilled Water	176.1±9.6	16.5±2.2	107.1
70% Methanol	456.1±11.7	278.4±21.6	30.1
70% Ethanol	418.0±34.4	212.3±14.6	43.7
Ascorbic Acid	-	-	6.3

Each value in the table is represented as Mean ± SD (n = 3)

Antioxidant activity

The antioxidant property of the extracts generated was assessed by the DPPH free-radical scavenging method which is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. The color of DPPH solution (purple) changes to yellow in presence of antioxidant was measured spectrophotometrically [28]. The results for prepared extracts are shown in Figure 1, respectively. The investigation showed that all the prepared extracts have ability to scavenge free radicals. The antioxidative capacity increases with increase of concentration of all extracts (at concentrations ranging from 1 - 200 µg/ml). Among all extracts, 70% methanol extract showed the most potent (IC_{50}- 30.1 µg/ml) antioxidant potential although this is significantly less as compared to standard (ascorbic acid; IC_{50}- 6.3 µg/ml). The IC_{50} DPPH values, the amount of total phenol and flavonoid content for all extracts are given in Table 2.

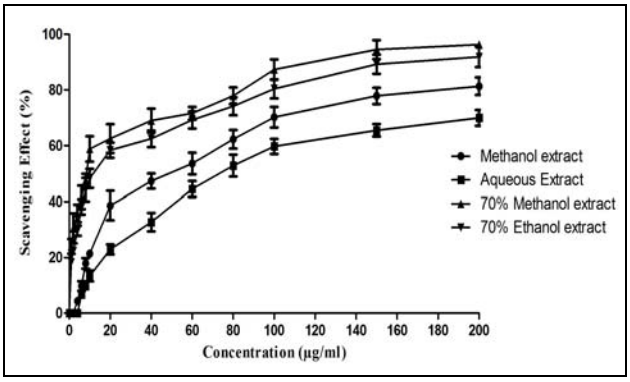

Fig 1: Antioxidant (DPPH scavenging) potential of methanol extract, aqueous extract, 70% methanol and 70% ethanol of outer skin of onion. The data are expressed as mean ± SD; n=3

It has been well documented that extracts with high phenolic content showed high antioxidant activity [35]. The present study revealed that 70% methanol extract has prominent antioxidant activity which could be attributed to the observed high phenol and flavonoid content.

Conclusion

In the present study, the effect of different solvents on extraction of total phenol and flavonoids from onion waste was investigated. It was shown that 70% methanol was the most appropriate solvent for extraction from outer scales of onion. A direct relation between the antioxidant activity and the polyphenolic content of the extract was observed. Thus, the use of methanol (70%) for recovering antioxidants from onion waste is very appealing because of its low cost which allows its use in the food industry and reduces the cost of the process.

Acknowledgement

The authors are deeply grateful to the University Grants Commission (UGC), New Delhi, India (No. F.7-265/2009(BSR)) for providing support and financial assistance to carry out this research.

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