

# Characterization of Antioxidant Quercetin Extracted from Onion skins and Radish leaves using Microwave and Conventional Extraction Methods

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## ABSTRACT

This research focused on characterization, identification of previously extracted Quercetin from two identified vegetable waste (Onion skin, Radish leaves). The Free Radical Scavenging activity with respect to Ascorbic acid was examined. Extraction was done by three methods i.e. Microwave, Soxhlet and Maceration, after that purification was done with the help of column chromatography and quantification of Quercetin performed by high performance liquid chromatography. Structural characterization of purified Quercetin was carried out in FTIR spectroscopy and DPPH method was used for determination of antioxidant activity of Quercetin. Yield of Quercetin (14.44µg/g) and antioxidant activity (87.58%) was found maximum for Onion skin extract with 90% Ethanol (EtOH) by using microwave at 400W as compared to Radish leaves. FTIR confirms the purity and structure of isolated Quercetin. Onion skin showed higher amount of Quercetin with microwave extraction which has good antioxidant activity.

*Key words: Antioxidant, Onion skins, Quercetin, Radish leaves and Extraction*

## Introduction

Antioxidants are essential in preventing unfavorable alterations in the flavor and nutritional value of food. Antioxidants guard cells from tissue damage brought on by a variety of human disorders (Shahidi *et al.*, 1992). Although synthetic antioxidants are frequently employed as food additives, their use has received criticism due to potential harmful or cancer-causing byproducts produced by their breakdown (Namiki, 1990). As a result, the investigation for endogenous protective substances in recognized foods has increased because their use just demands

for altering food formulations. Several researchers mentioned the presence of beneficial phytochemicals with antioxidant activity from some of the vegetable waste. On an industrial basis, some vegetable wastes have been investigated for this reason as sources of potentially safe and stable natural antioxidants, such as Polyphenols (Speisky *et al.*, 2012).

During the past ten years, commercial Onion farming, the second-largest horticultural crop in the world, has expanded by more than a quarter of total production, and is now anticipated to produce between 66 and 85.7 million tons year (Campone *et al.*,

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2018). Onion, pharmacologically known as *Allium cepa*, is one of the largest sources of Quercetin and its conjugates, as well as organosulfur compounds and pigments like Anthocyanin. It has been shown that the non-edible outer skins of the Pearl, Red, Yellow, and White Onion cultivars contain six times more phenolics than the corresponding edible Onion flesh (Albishi *et al.*, 2013). Because of its distinctive smell and the quick development of phytopathogenic agents, vegetable processing byproducts such as Onion peel is not employed as feed or organic fertilizer (Benitez *et al.*, 2011), making it a financially appealing source of free radicals. Onion skin is a good source of dietary fiber and phenolic chemicals, though. Quercetin, a potent antioxidant, is also abundant in the skin.

In instance, the root vegetable Radish (*Raphanus sativus* L.), which belongs to the *Cruciferaeae* family, is a significant vegetable crop grown around the world (Tsouvaltzis and Brecht, 2014). The components of radish were discovered to provide significant therapeutic and dietary benefits. Hence, it was proposed as a complementary therapy for a number of conditions, such as cancer, hyperlipidemia, and coronary heart disease (Curtis, 2003). According to research, certain sections of *Raphanus sativus*, popularly known as Radish, contain secondary metabolites like Terpenoides, Coumarins, flavonoids, Anthocyanins, Isothiocyanates, Saponins, Alkaloids, Ascorbic acid, Folic acid, and Potassium that may have therapeutic advantages. Flavonoids like Quercetin, Kaempferol, Myricetin, and Apigenin are abundant in radish. Radish leaves and stems have been shown to contain polyphenolic compounds including Quercetin and other compounds.

Fresh produce (fruits and vegetables), such as red Onions, Apples, and Berries, contain the secondary metabolite Quercetin, a carbohydrate free flavonoid. Yellow powdered Quercetin has a distinct odor and flavor that is bitter. One of the most researched plant flavonoids, Quercetin has been linked to a number of health benefits, including antioxidant, anticancer, anti-inflammatory, antiaggregatory, antihyperten-

sive, and neuroprotective properties. The uneatable portion of onions contains more than 77 times more Quercetin than that of the edible portion (Kang *et al.*, 1998). This research aims at characterization of Quercetin purified from Onion skins and Radish leaves extracted by three extraction methods (Microwave Assisted Extraction, Soxhlet and Maceration).

## Materials and Methods

### Materials

Onion skins and Radish leaves were purchased from local vegetable market in Prayagraj, Uttar Pradesh. The wastes were collected, washed, dried and stored in powdered form in polyethylene bags at 4°C.

Ethanol, Methanol, Ascorbic acid, Quercetin, Ethyl Acetate, n-Hexane, Toluene were used for extraction and analysis purpose. All the chemicals used were of analytical grade.

### Preparation of Crude Extract

Fifteen grams of powdered sample and 100 ml of Ethanol concentrations were used to prepare crude extracts. Three extraction methods were used for preparation of extracts. Table 1 shows the extraction methods and their parameters.

### Quantification of Quercetin

Weighed and transferred 10mg each of standard Quercetin and crude extract in to 100 ml volumetric flask and added about 50ml of diluent (Methanol). Filtered and degassed Methanol and Water were mixed in the ratio of 70: 30 and was used as mobile phase. The solution was sonicated for 5min and cooled to room temperature and made it up to volume with diluent. Blank diluent, standard solution and test sample solution were injected into the column individually. Chromatographic conditions are given in Table 2 and recorded the chromatogram at 228nm (ICH 2005).

**Table 1.** Extraction methods and their parameters

Extraction methods	Ethanol concentration	Parameters	Time
MAE	30%, 60%, 90%	200W, 300W, 400W	5 min
Soxhlet	30%, 60%, 90%	60°C	8 h
Maceration	30%, 60%, 90%	Room temperature	24h

MAE: Microwave Assisted Extraction

**Table 2.** Chromatographic conditions

Variable	Conditions
Mobile phase	Methanol : Water (70:30)
Column (Shimadzu)	C 18, 250x4.6mm, 5µm
Wavelength	228 nm
Injection volume	20 µl
Flow rate	1.0 ml/min
Oven temp.	Ambient
Run time	15 min

**Purification of Quercetin**

Purification of Quercetin was done with the help of Column Chromatography. Stationary phase was prepared with silica gel and ethyl acetate and the blank column was eluted with ethyl acetate for 10 min. 5 g of extracts were placed on the top of packed stationary phase. Elutions were started with n-Hexane: Ethyl Acetate (80:20) and increased with solvent polarity in the ratio of 70:30 to 10:90 at the flow rate of 10ml/min.

**Identification of Quercetin**

Identification of collected fractions was done with the help of Thin Layer Chromatography. Collected fractions from Column Chromatography were subjected to the TLC plates (10x6 cm) with the help of capillaries and the solvent system used for TLC was toluene: ethyl acetate: methanol (4:0.5:0.5). The retardation factor ( $R_f$  value) were calculated for isolated sample and compared with coinciding standard.  $R_f$  values of the spot was calculated using the formula as shown in Equation 1. Identified fractions were concentrated in rotary evaporator.

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}} \quad \text{Equation 1}$$

**Characterization of Isolated Quercetin**

The structure of isolated Quercetin was elucidated by FTIR spectroscopy. 2µg of sample was placed in a holder in the path of IR source. Detector reads the

analog signal and converts the signal to spectrum. A computer was used to analyse the signals and identify the peaks and record the graph. FTIR spectra of the standard and isolated Quercetin were recorded in the range of 400 to 4000  $\text{cm}^{-1}$ .

**Antioxidant Activity of Isolated Quercetin**

Antioxidant activity of isolated Quercetin was done by DPPH method described as per Brand-Williams *et al.*, (1995) at different concentration ranging from 100 to 1000 µg/ml. The antioxidant activity or Free Radical Scavenging Activity (FRSA) was calculated using the formula shown in Equation 2.

$$FRSA = \frac{A_o - A_i}{A_o} \times 100$$

$A_o$  = Absorbance of control  
 $A_i$  = Absorption of sample

**Statistical Analysis**

Experiments were conducted in triplicate. Data were expressed as the means of these values ± the standard deviations (SD). Analysis of variance (ANOVA)- one way ANOVA was used to assess data.

**Results and Discussion**

Quercetin was detected in Onion skin and Radish leaves at the retention time in between 3.9 to 4.2 min by High Performance Liquid Chromatography. Quercetin yield of Onion skins was showed in Table 3. HPLC chromatograms of standard and samples are presented in Fig.1 and Fig.2. Quercetin yield was higher for microwave extraction at 400W with 90% Ethanol i.e. 14.44 µg/g and Soxhlet extraction showed lower Quercetin yield (0.257 µg/g) with 30% EtOH. Quercetin yield significantly increased with Ethanol concentration and power level. MAE heats all the sample fluid, allowing the extraction solution to reach the desired temperature more rapidly. It avoids the thermal gradient caused by con-

**Table 3.** Quercetin yield of Onion skin extract, quantify by using HPLC

EtOH conc.	Quercetin Yield in µg/g from Onion Skin			SE 60°C	M Room temp.
	200W	300W	400W		
30% EtOH	0.60±0.75	0.45±0.78	2.84±0.56	0.25±1.27	0.90±0.67
60% EtOH	1.45±0.93	4.45±0.46	3.75±25	0.65±0.84	3.36±0.39
90% EtOH	5.19±1.12	10.39±0.69	14.44±.48	2.81±0.33	

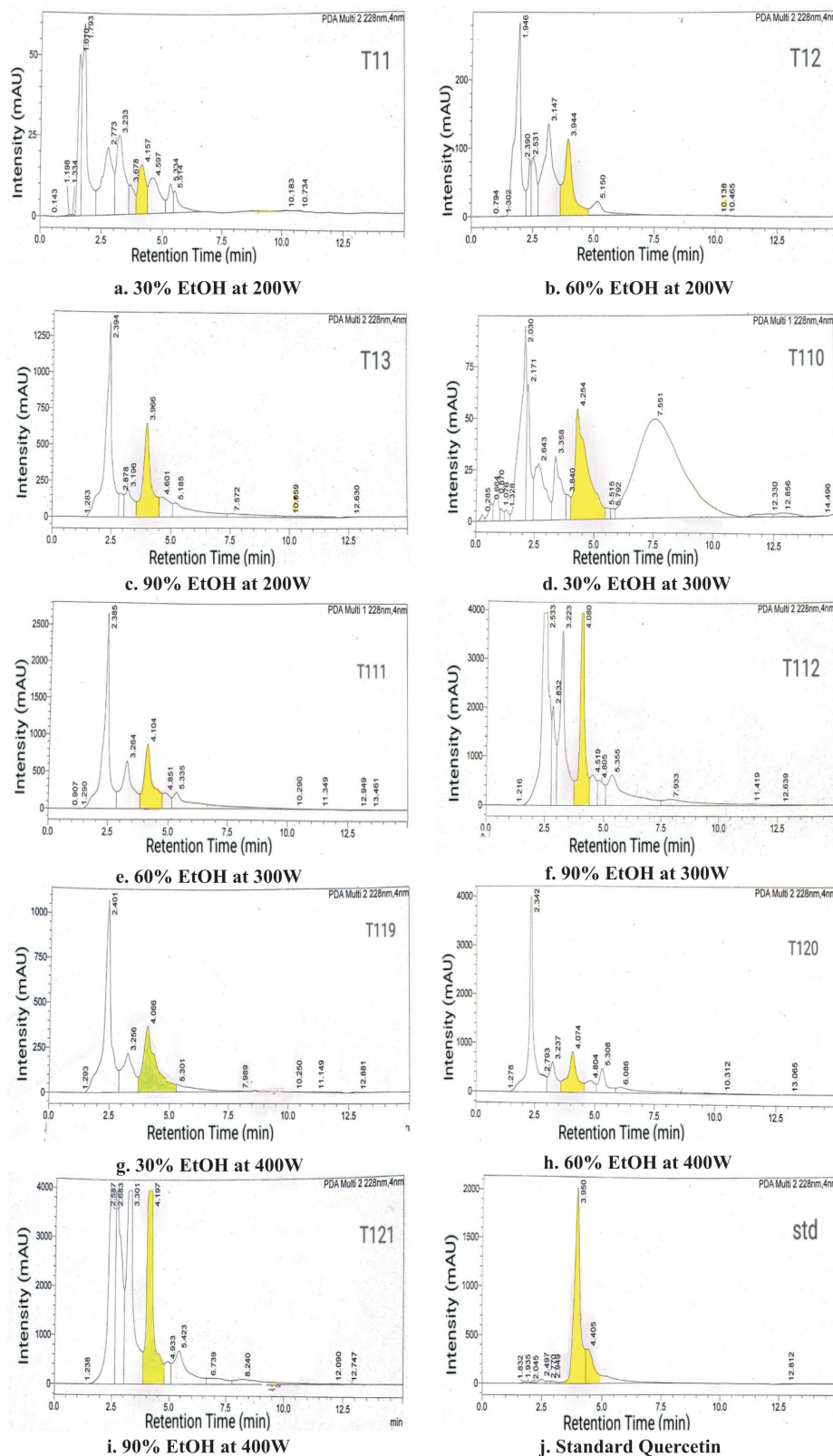


Fig. 1. Chromatogram of Onion skin extract (a-i) prepared by Microwave extraction and standard Quercetin (j)

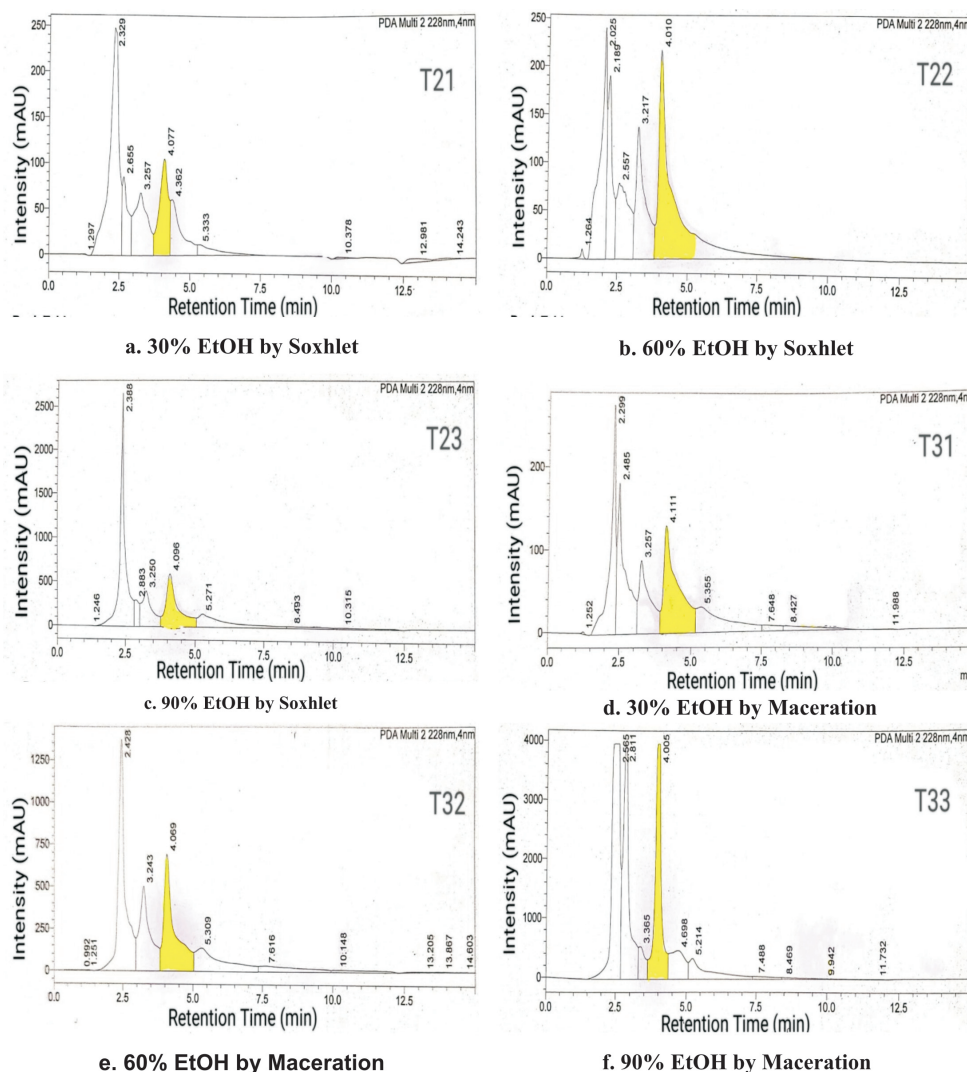


Fig. 2.(a-f) Chromatogram of Onion skin extract prepared by Conventional extraction

ventional heating (Biesaga, 2011), which increases the risk of degradation of thermolabile bioactive compounds (Wu and Chau, 2001).

Quercetin yield is showed in Table 4 for Radish leaves. Higher Quercetin (3.449 µg/g) was extracted from Microwave extraction with 90% EtOH at 400W and lower Quercetin obtained of 0.181µg/g from Maceration with 30% EtOH. Yield of Quercetin increased as solvent polarity and power level increased due to polar nature of Quercetin. Chromatograms of radish leaves extract were displayed in Fig. 3 and Fig. 4. Rupasinghe *et al.*, (2011) recorded lower Quercetin concentration, due to hydrolysis of glycosides and degradation the aglycone due to extended extraction period (Ewald *et al.*, 1999). Similar results

were reported by Sharifi *et al.*, (2017). Since most flavonols occur naturally in the glycosylated form, the acidification of solvents is not appropriate in terms of determining the distribution and concentration of naturally occurring flavonols in plant tissue (Tsao *et al.*, 2003).

Extracts of Onion skin, Radish leaves were loaded to Column Chromatography. The column was eluted successively, using n-Hexane and Ethyl Acetate gradient. Onion skin, Radish leaves extract pooled out 5 and 3 fractions of Quercetin respectively. From the TLC analysis, Quercetin was identified. It showed spots which coincided with that of the reference Quercetin.  $R_f$  value for standard Quercetin was 0.5 which was approximately similar with

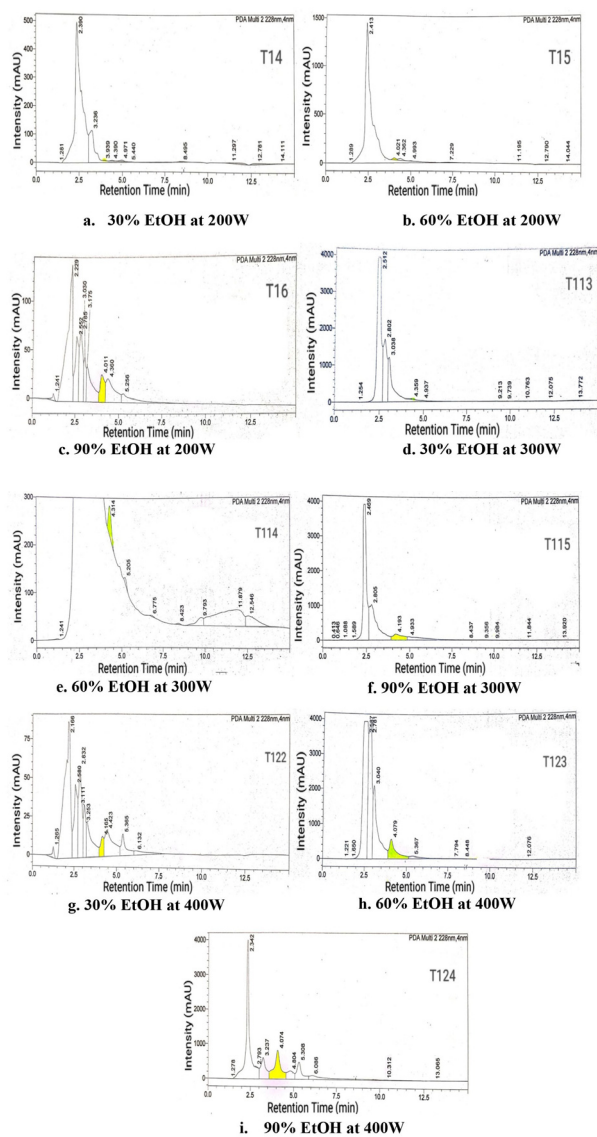


Fig. 3. (a-i) Chromatogram of Radish leaves extract prepared by Microwave Extraction

Quercetin isolated from samples which confirms the purity of isolated compound. Quantity of isolated Quercetin is given in Table 5. After confirmation above fractions was pooled together and concentrated under vacuum. On drying fractions yielded yellow colored powder, gave positive test for Quercetin. Onion skin extract gave the highest quantity of Quercetin.

The isolated Quercetin was structurally characterized using FTIR spectroscopy methods. The FTIR spectrum of Quercetin standard is presented in Fig. 5. FTIR spectrum of isolated Quercetin is displayed in Fig. 6, Fig. 7. The broad bands at 3392.79  $\text{cm}^{-1}$ ,

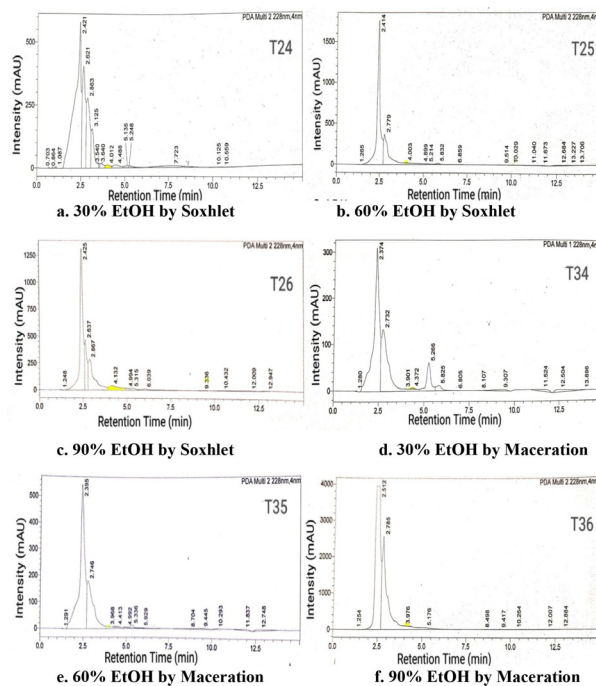


Fig. 4. (a-f) Chromatogram of Radish leaves extract prepared by Conventional Extraction

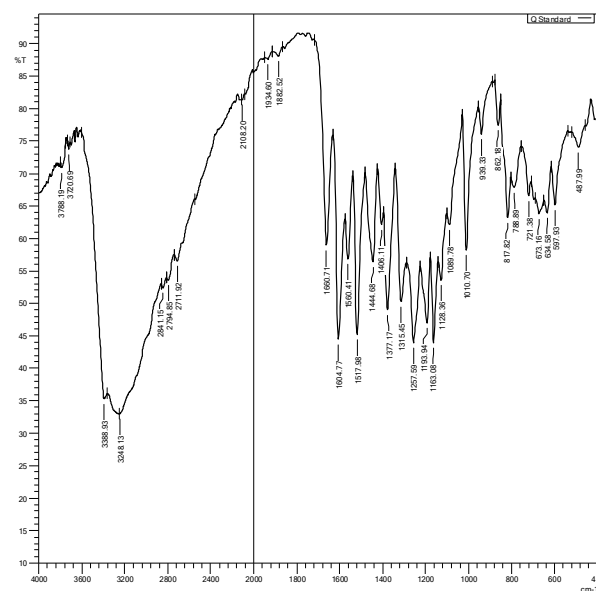


Fig. 5. FTIR Spectra of Standard Quercetin

Table 5. Quantity of isolated Quercetin from extracts

Extracts	Isolated Quercetin Quantity (mg)
Onion Skins	170
Radish Leaves	160

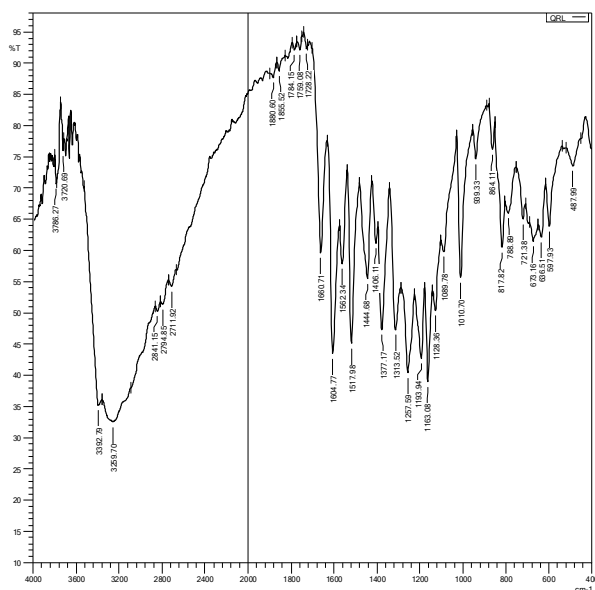


Fig. 6. FTIR Spectra of Isolated Quercetin from Radish leaves

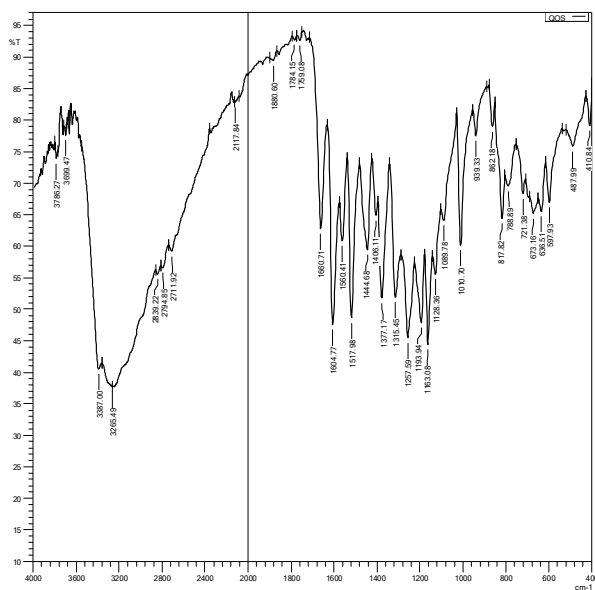


Fig. 7. FTIR Spectra of Isolated Quercetin from Onion skins

3388.93  $\text{cm}^{-1}$  and 3265.49  $\text{cm}^{-1}$  originate from the valence vibrations  $\nu(\text{O-H})$ . The intensive band at 1784.15  $\text{cm}^{-1}$  is the result of valence vibration of free ketone group ( $\text{C=O}$ ) (Pralhad and Rajendrakumar, 2004). The band at 1759.08  $\text{cm}^{-1}$  and 1728.22  $\text{cm}^{-1}$  belongs to the  $\nu(\text{C=O})$  vibration. The absorption peaks positioned at 1604.77  $\text{cm}^{-1}$  and 1660.71  $\text{cm}^{-1}$  are assigned to the ( $\text{C=C}$ ) aromatic ring stretch. Alkyne ( $\text{C-C}$ ) aromatic ring stretching vibrations are noticed at 1560.41  $\text{cm}^{-1}$ , 1517.98  $\text{cm}^{-1}$ , 1406.11  $\text{cm}^{-1}$  and 1444.68  $\text{cm}^{-1}$ . In the spectrum, band of vinylidene ( $\text{C-H}$ ) plane bend at 1377.17  $\text{cm}^{-1}$ , 1315.45  $\text{cm}^{-1}$ , 1257.59  $\text{cm}^{-1}$  and 1255.66  $\text{cm}^{-1}$  and the lower peaks absorbance at 939.33  $\text{cm}^{-1}$ , 862.18  $\text{cm}^{-1}$  and 821.68  $\text{cm}^{-1}$  were observed. Aromatic ( $\text{C-H}$ ) bending out of plane vibrations were also assigned at 2839.22  $\text{cm}^{-1}$ , 1010.7  $\text{cm}^{-1}$ , 721.38  $\text{cm}^{-1}$ , 673.16  $\text{cm}^{-1}$ , 636.51  $\text{cm}^{-1}$  and 634.58  $\text{cm}^{-1}$  peaks positions.  $\text{C-CO-C}$  stretching and bending vibrations of ketones were noticed at 1163.08  $\text{cm}^{-1}$  and 1128.36  $\text{cm}^{-1}$ , which confirms that the isolated compound is Quercetin. Results were agreement with Chaurasiya *et al.*, (2012) for molecular structure of Quercetin.

The percent Free Radical Scavenging potential was higher for Quercetin isolated from Onion skin of 85.58%. The antioxidant activity of the isolated Quercetin with respect to the standard molecule

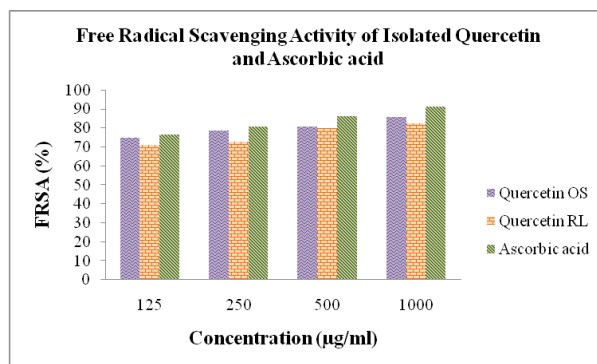


Fig. 8. Free Radical Scavenging Activity of isolated compound Quercetin compared with Ascorbic acid standard

Table 4. Quercetin yield of Radish leaves extract, quantify by using HPLC

EtOH conc.	Quercetin Yield in $\mu\text{g/g}$ from Radish Leaves				
	200W	MAE 300W	400W	SE 60°C	M Room temp.
30% EtOH	0.183±0.12	0.248±1.05	0.259±0.59	0.188±0.42	0.181±0.91
60% EtOH	0.250±0.56	0.344±0.98	2.568±0.86	0.237±1.56	0.182±0.52
90% EtOH	0.367±0.34	0.516±0.43	3.449±0.46	0.237±1.23	0.236±0.37

(ascorbic acid) was calculated as displayed in Fig. 8. The decrease in the absorbance of the DPPH radical with respect to ascorbic acid is caused by antioxidant activity through the reaction between antioxidant Quercetin and free radical results in the scavenging of radical by hydrogen donation (Bukhari *et al.*, 2008). Same parameters were observed by Zahoor *et al.*, (2018) for Quercetin isolated from *Aesculusindica* fruit.

## Conclusion

From the present study, antioxidant Quercetin has been extracted and purified successfully from onion skin and radish leaves. Spectrum of standard Quercetin shows similarity with isolated Quercetin which confirms the purity of isolated compounds. For extraction three methods were used to prepare crude extract but microwave extraction gave higher Quercetin yield and solvent concentration play a major role in extraction process. Quercetin is highly soluble in 90% Ethanol concentration. Among both of wastes onion waste gave higher Quercetin yield and antioxidant activity. Quercetin isolated from onion skin can be a good replacement of synthetic antioxidant.

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## Conflict of interests

The authors declare that there is no conflict of Interest.

## References

- Albishi, T., John, J.A., Al-khalifa, A.S. and Shahidi, F. 2013. Antioxidative phenolic constituents of skins of onion varieties and their activities. *Journal of Functional Foods*. 5 (3): 1191, 1203.
- Benitez, V., Molla, E., Martin-Cabrejas, M.A., Aguilera, Y., Lopez-Andreu, F.J., Cools, K. and Esteban, R.M. 2011. Characterization of Industrial Onion Wastes (*Allium cepa* L.): Dietary Fibre and Bioactive Compounds. *Plant Foods Human Nutrition*. 66 (1): 48–57.
- Biesaga, M. 2011. Influence of extraction methods on stability of flavonoids. *Journal of Chromatography A*. 1218 (18): 2505-2512.
- Brand-Williams, W., Cuvelier, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT- Food Science and Technology*. 28 (1): 25-30.
- Bukhari, S.B., Bhangar, M.I. and Memon, S. 2008. Antioxidative activity of extracts from fenugreek seeds (*Trigonellafoenum-graecum*). *Pakistan Journal of Analytical and Environmental Chemistry*. 9 (2): 78-83.
- Campone, L., Celano, R., Piccinelli, A. L., Pagano, I., Carabetta, S., Di Sanzo, R., Russo, M., Ibañez, E., Cifuentes, A. and Rastrelli, L. 2018. Response Surface Methodology to optimize supercritical carbon dioxide/co-solvent extraction of brown onion skin by-product as source of nutraceutical compounds. *Food Chemistry*. 269 (7): 495-502.
- Chaurasiya, A., Upadhyay, A. and Shukla R.N. 2012. Isolation of Quercetin from the leaves of *Azadirachta indica* and antidiabetic study of the crude extracts. *Journal of Pharmaceutical and Biomedical Sciences*. 25 (25): 179-181.
- Curtis, I. S. 2003. The noble radish: Past, present and future. *Trends in Plant Science*. 8 (7): 305–307.
- Ewald, C., Fjellkner-Modig, S., Jahansson, K., Sjöholm, I. and Akesson, B. 1999. Effect of processing on major flavonoids in processed onions, green beans, and peas. *Food Chemistry*. 64 (2): 231-235.
- ICH, 2005. International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use, harmonized triplicate guideline on validation of analytical procedures: text and methodology Q2 (R1), complementary guideline on methodology incorporated in November 2005 by the ICH steering committee, IFPMA, Geneva.
- Kang, S., Kim, Y., Hyun, K., Kim, Y., Song, B., Shin, S. and Park, Y. 1998. Development of separating techniques on Quercetin-related substances in onion (*Allium cepa* L.) content and stability of Quercetin-related substances in onion. *Journal of the Korean Society of Food Science and Nutrition*. 27(4): 682-686.
- Namiki, M. 1990. Antioxidants/Antimutagens in food. *Critical Reviews in Food Science and Nutrition*. 29(4): 273-300.
- Pralhad, T. and Rajendrakumar, K. 2004. Study of freeze-dried Quercetin cyclodextrin binary systems by DSC, FT-IR, X-ray diffraction and SEM analysis. *Journal of Pharmaceutical and Biomedical Analysis*. 34 (2): 333-339.
- Rupasinghe, H.P.V., Kathirvel, P. and Huber, G.M. 2011. Ultrasonication-assisted solvent extraction of quercetin glycosides from 'Idared' apple peels. *Molecules*. 16 (12): 9783-9791.
- Shahidi, F., Janitha, P.K. and Wanasandura, P.D. 1992. Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition*. 32(1): 67-103.



- Sharifi, N., Mahernia, S. and Amanlou, M. 2017. Comparison of different methods in quercetin extraction from leaves of *Raphanus sativus* L. *Pharmaceutical Sciences*. 23 (1): 59-65.
- Speisky, H., Lopez-Alarcon, C., Gomez, M., Fuentes, J. and Sandoval-Acuna, C. 2012. First web-based database on total phenolics and oxygen radical absorbance capacity (ORAC) of fruits produced and consumed within the south Andes region of South America. *Journal of Agriculture and Food Chemistry*. 60 (36): 8851-8859.
- Tsao, R., Yang, R., Young, C. and Zhu, H. 2003. Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). *Journal of Agriculture and Food Chemistry*. 51 (21): 6347-6353.
- Tsouvaltzis, P. and Brecht, J. K. 2014. Changes in quality and antioxidant enzyme activities of bunched and topped radish (*Raphanus sativus* L.) plants during storage at 5 or 10°C. *Journal of Food Quality*. 37 (3): 157-167.
- Wu, J. and Chau, F. 2001. Ultrasound-assisted extraction of ginseng saponins from ginseng roots and cultured ginseng cells. *Ultrasonics Sonochemistry*. 8 (4): 347-352.
- Zahoor, M., Shafiq, S., Ullah, H., Sadiq, A. and Ullah, F. 2018. Isolation of quercetin and mandelic acid from *Aesculus indica* fruit and their biological activities. *BMC Biochemistry*, 19 (5): 1-14.
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