International Journal of Food Science and Nutrition
ISSN: 2455-4898; Impact Factor: RJIF 5.14
Received: 06-11-2019; Accepted: 09-12-2019
www.foodsciencejournal.com
Volume 5; Issue 1; January 2020; Page No. 27-32

Selection and optimization of bivalent solvent system for the extraction of phenolic compounds from pomegranate peel powder

Andhale Shubham[1], Bhoite Anjali[2*]
[1] Research Scholar, Department of Food safety, Quality and Nutrition, MIT college of food technology, MIT ADT University, Pune, Maharashtra, India
[2] HOD, Associate professor, Department of Food safety, Quality and Nutrition, technology, MIT college of food technology, MIT ADT University, Pune, Maharashtra, India

Abstract
Present study was carried out to optimize the phenolic compound extraction of pomegranate peel in binary solvent system. ethanol ether and ethanol: water are two binary solvent systems used for extraction of phenolic compounds with potent antioxidant activity. Six different combinations of these two binary solvent systems were used for the extraction of phenolic compounds from pomegranate peel, appropriate bivalent solvent system ethanol: ether (70:30) was reported with Phenolic compounds with potent antioxidant activity (1783 mg/L of GAE phenolic content and 87.58% DPPH inhibition). In order to optimizes the extraction conditions for selected bivalent solvent system, one factor at a time study was carried out to determine the effect of time, temperature, rpm and concentration of peel powder on the total phenolic content and antioxidant activity. Studty reveals the 125 rpm at 37 ºC for 6 hour is the best condition for obtaining the phenolic content with enhanced antioxidant activity for selected binary solvent system.

Keywords: total phenolic content, antioxidant activity, rpm, temperature, time

1. Introduction
Oxidation is the main cause of deterioration of foods and it considerably limits the shelf life of these products (Nerín *et al*. 2008) [1] because of which food industry are always in search of new antioxidant compounds. Antioxidant application in food packaging material is very common. However the use of chemical antioxidants Nowadays, especially due to consumers' demands, there is a tendency to search for natural AO molecules that are not related to possible toxicological side-effects, e.g. suspected carcinogenic potential (Imaida *et al*. 1983; Chen *et al*. 1992) [2,3] therefore natural antioxidants are of great interest because of safety and low toxicity. Among the fruits Pomegrate being rich in phenolic a compound and antioxidant activity is of great concern. The peel of pomegranate especially reported with rich source of natural antioxidant is selected in present study to extract the natural antioxidant compounds. The significant influence on the content of antioxidant activity is mainly because of extraction solvent, time and temperature and interaction of these factors. Many studies have shown that solvent extraction power is one of the significant out of these. Antioxidant Activity is mainly attributed to the phenolic compounds present in the peels of various fruits, based on which so many scientist have made the attempt to extract these phenolic compound in various solvents andeven tried to applied in food products for better formulation of antioxidant enriched food product, which is as per the consumer perception. It was pointed out that from a practical view point, a suitable extracting procedure should be developed to recover as many antioxidants as possible to produce extracts rich in natural antioxidants for potential application in health-promoting supplements for the food industry (Y. Li, C *et al.*, 2006) [4] Considerable efforts have been made to extract and characterize the pomegranate bioactive compounds (Gil *et al.*, 2000; Kulkarni and Aradhya 2005 [5, 6] however so far, work on the screening and selection of best solvent and/or solvent combination to obtain highest antioxidant activity and phenolic compound is lacking.phenolic are often extracted in higher amounts in more polar solvents such as aqueous methanol/ethanol as compared with absolute methanol/ethanol (Siddhuraju, P and Becker, K. 2006, Sultana, B *et al.*, 2007). [7,8] The peel phenolic compounds can be extracted using various improved extraction method like, Ultrasound assisted extraction (UAE); Microwave assisted extraction (MAE), Supercritical fluid extraction (SFE) and Subcritical Water extraction (SWE) all these methods being a expensive and not always radially aviable the solvent extraction still has been widely used to extract phenolic compounds from fruits and vegetables. Among all the investigated variables (pre-treatment of the sample, solvent/sample ration, type of solvent, time and temperature of extraction) to ensure the efficiency of extraction, type of solvent has been the most studied factor. Polarity of solvents play a vital role in extraction process since with change in solvent polarity its ability to dissolve especial group of antioxidant compounds alters and influences the antioxidant activity estimation. It is impossible to develop a universal solvent that is suitable for the all kinds of antioxidant compounds extraction from plants because plant materials have diverse chemical profile. Thus, screening process is important to justify the best solvent in antioxidant compounds extraction so that the maximum antioxidant activity for a certain sample could be identified. The present study is therefore conducted with the objective to investigate the most effective binary

solventsystem and its optimizationfor extracting the potent antioxidant compounds, especially potentantioxidants from pomegranate

Materials and method

Raw Material and Sample preparation (Peel drying and powder)

The fresh fruits of Pomegranate *cv*.Bhagva, varietywas obtained from farmers of Solapur district region of Maharashtra and peel manually to obtain the fresh peels.Pomegranate fruits were washed with potable water and peel was separated manually using peeler and dried in tray dryer at 50 ^{O}C for 48 hours. The dried peels where ground with pestle and mortar to coarse powder and finally ground in to fine powder (up to 1mm size)using grindner Powder was stored in a refrigerator at 4^{O}C until further use. (Shalini Malviya *et al*.2014) [9].

Extraction

The three different solvents with different proportion of six combination (30% ethanol: 70% water, 50% ethanol: 50%water, 70%ethanol: 30%water, 30% ethanol: 70% di-ethyl ether, 50% ethanol: 50% di-ethyl ether, 70% ethanol: 30% di- ethyl ether) were used for extraction.The 20 g of sample sample (1:5 ratio of sample and solvent) were prepared and extraction was carried out at at 37^{o}C for 24 hours using shaking incubator at the constant speed 125 rpm. The filtered samples (using Whatman no. 1 filter paper) were evaporated at 70 ^{o}C for 6 hours in water bath and concentrate was stored in regenerator at 4 ^{0}Cuntil further use. (Shalini Malviya *et al*.2014) [9].

Assessment of bioactive compounds

Total phenolic compounds were determined by Folin-Ciocalteu (FC) colorimetric method at 765 nm as describe by Singleton and Rossi (1965) [10]. The results were expressed as mg of Gallic acid equivalent (GAE) per litre of the sample.Antioxidant activity was determined by performing FRAP assay reported by (Benzie,I.F.F. and J.J. Strain 1996) [11]with slight modifications and values were quoted as AAE per litre of sample.Free radical scavenging activity (% inhibition) using DPPH assay was determined as per the (Siddhuraju *et al.,* 2002) [12] and DPPH % inhibition was calculated.

Optimization study

One factor at a time (OFAT) interaction study was performed for various parameters like time, temperature, peel concentration and rpm as per the guidelines given by (Kheiralla *et al*., 2018) [13].

Stastical analysis

Experimental data was analysed for analysis of variance (ANOVA) and differences between means were assessed by Duncan's new multiple range test at the significance defined $p \leq 0.001$using SAS 9.3 software.

Result &Discussion

Selection of solvent for extraction

Research pertaining to the solvent extraction of phenolic compounds from plants have focused on methanol solvent as a best solvent in capacity of maximum extraction of plant phenolics, moreover aqueous solution of these solvents has also proven best for the phenolic extraction. In the advancement of solvent extraction last few years studies have also shown the application of binary solvent system could be the best for extraction. The variation in the yield of phenolic compounds is depends upon the solvent used for extraction. The selection of better solvent system is still in scope The variety of solvents and combination of solvents as bivalent system thus still having scope for the extraction of phenolic compounds..

Table 1: Optimization of bivalent solvent system for phenolic compounds offering high Antioxidant yield

Sr. no.	Solvents used for extraction	Total phenol mg/100ml of gallic acid	DPPH % inhibition
1	30% ethanol: 70% water	171.8^{b}	88.13^{a}
2	50% ethanol: 50% water	184.4^{a}	87.54^{a}
3	70% ethanol: 30% water	180.7^{a}	88.96^{a}
4	30% ethanol: 70% ether	173.2^{b}	87.34^{a}
5	50% ethanol: 50% ether	177.3^{b}	87.82^{a}
6	70% ethanol: 30% ether	178.3^{b}	87.58^{a}

a and b shows significant difference

In present study attempt was carried out to quantify the phenolic content in the two binary solvent system of food grade solvents, ethanol and ether. The content of total phenolic compounds in the pomegranate peel extracts using two binary solvents was analyzed and data is presented in table no. 1 reveals that there is a significant difference in phenolic content if ethanol is used along with water in various proportion than that of ethanol is used in various combination with diethyl ether. The less amount of phenolics were extracted when ethanol is used in combination with diethyl ether because of the polar nature of the phenolics which limits their extraction in ethanol diethyl ether solvent (Shiban, M.S. *et al.,* 2012.) [14] Ethanol–water bivalent solvent is found suitable for the extraction because of different polarity of both the solvent, water is polar and ethanol is mid polar leads the high amount of phenolic extraction (Zhang, Z.S. 2007) [15]. However this study reveals though there is presence ofless amount of phenolics, %DPPH inhibition not significantly different in all the samples. Bivalent solvent of ethanol and diethyl ether modulate the polarity and, thus favour the solubility of hydrolysable tannins such as punicalagin, ellagic acid and gallic acid. This is also because of antioxidant activity was strongly dependent on the solvent due to the different antioxidant potentials of compounds with different polarity (Julkunen-Tiito, R 1985, Marinova, E.M 1997) [16, 17]. Results for the ethanol: ether binary solvent supports the findings of Bajpai, M *et al*., 2005 and Ruanma, K *et al*., 2010 [18, 19] who stated no correlation between phenolic content and antioxidant activity. Punicalagin, ellagic acid and gallic acid are main phenolic content and responsible for high antioxidant activity. Hydrolysable tannins are phenolic compounds which contain a central core of glucose or another polyol esterified

with gallic acid (gallotannins) or with hexahydroxydiphenic acid (ellagitannins).(Singh, M *et al*., 2014) [20] Punicalagin isomers are part of family ellagitannin which after hydrolysis in aqueous solution release ellagic acid (Gil *et al*., 2000) [21] best extraction of these phenolic compounds were reported by Abid, M *et al.*, 2017 [22] the high yield of Punicalagin, ellagic acid and gallic acid in ethanol: ether system. Therefore In present study the ethanol ether bivalent solvent system in various proportion was studied for better yield of antioxidants and 70: 30 proportion of ethanol and ether was found to contain good amount of antioxidants hence, further this solvent system is selected for optimization study.

Table 2: One factor AT Time interaction for various parameters (time, temperature, concentration and RPM)

Parameter		Peel (%)								
		1%			2%			3%		
		Time (hours)								
	Temp ^{o}C	2	4	6	2	4	6	2	4	6
Total phenol content (mg/L of GAE)	30	757.3^{d}	944^{d}	950.66^{d}	1331^{a}	1309.66^{a}	1341^{a}	815.22^{c}	1358.6^{a}	1409.33^{a}
	35	864^{d}	862^{d}	983.33^{d}	1171.33^{c}	1219^{c}	1181^{cd}	1416.33^{a}	1345.6^{a}	1519.33^{a}
	37	918.6^{c}	857.33^{c}	910 ^{d}	1379.33^{ab}	1283.66^{a}	1374.33^{a}	1276^{ab}	1418.6^{a}	1414.33^{a}
	40	1195.33^{b}	1425^{a}	1417.33^{a}	1447^{a}	1382^{a}	1487.66^{a}	1479.5^{a}	1523.6^{a}	1555.66^{a}
Antioxidant activity (mg/100 ml of AAE)	30	32.09^{e}	31.11^{e}	29.84^{e}	32.05^{e}	31.21^{e}	20.08^{f}	31.52^{e}	31.00^{e}	30.12^{e}
	35	30.71^{b}	32.24^{b}	32.10^{b}	31.16^{b}	30.56^{b}	31.24^{b}	32.19^{b}	31.16^{b}	33.34^{b}
	37	34.89^{cd}	33.48^{d}	32.63^{d}	36.00 ^{c}	34.09^{d}	32.47^{d}	38.24^{b}	34.91^{cd}	33.43^{d}
	40	30.01^{e}	36.59^{a}	32.58^{b}	41.81^{a}	42.23^{a}	36.36^{a}	36.75^{a}	38.61^{a}	33.92^{b}

a and b shows significant difference

The selected bivalent solvent ethanol and ether (70:30) proportion was further optimized by one –one factor interaction study. The temperature, time and peel powder concentration were the selected independent variable for the optimization while the total phenolic yield and antioxidant activity were the dependent variable for present study. Temperature ranges 30^{o}C, 35^{o}C, 37^{o}C and 40^{o}C were studied at time interval 2, 4 and 6 hrs., when the concentration of pomegranate peel was 1%, 2% and 3%.Total 36 trails were performed and after quantification of phenolic content and antioxidant activity in all samples effect of each variable on phenolic content and antioxidant activity were well studied to reveal the optimum conditions.

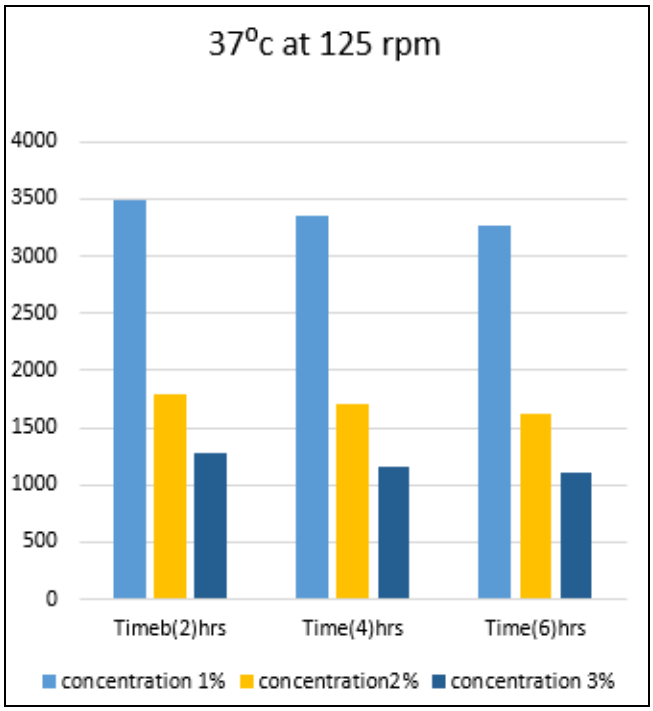

Fig 1: total phenolic extraction at 37 ºC for 125 rpm for different concentration of peel powder.

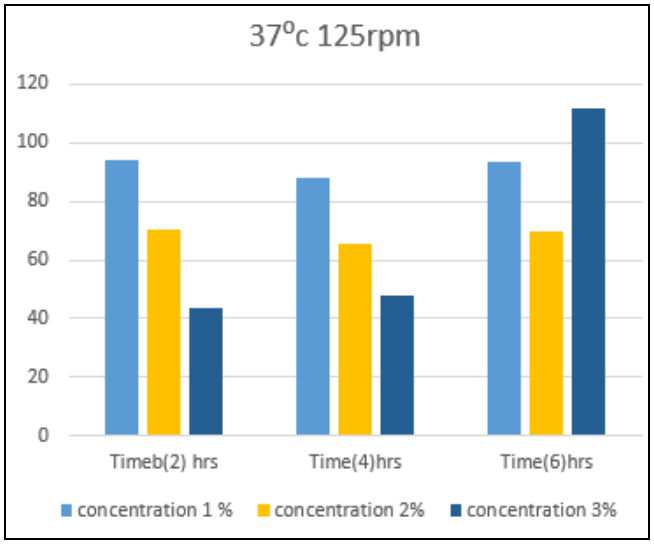

Fig 2: antioxidant activity at 37 ºC for 125 rpm for different concentration of peel powder

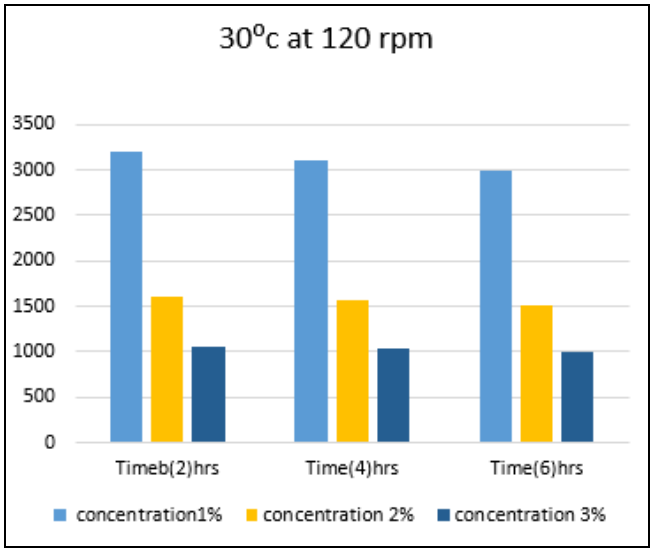

Fig 3: Total phenolic extraction at 30 ºC for 120 rpm for different concentration of peel powder.

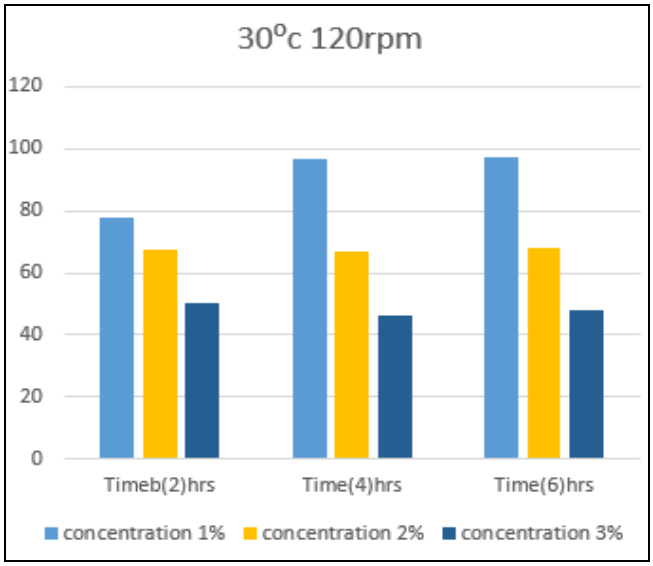

Fig 4: antioxidant activity at 30 ºC for 120 rpm for different concentration of peel powder

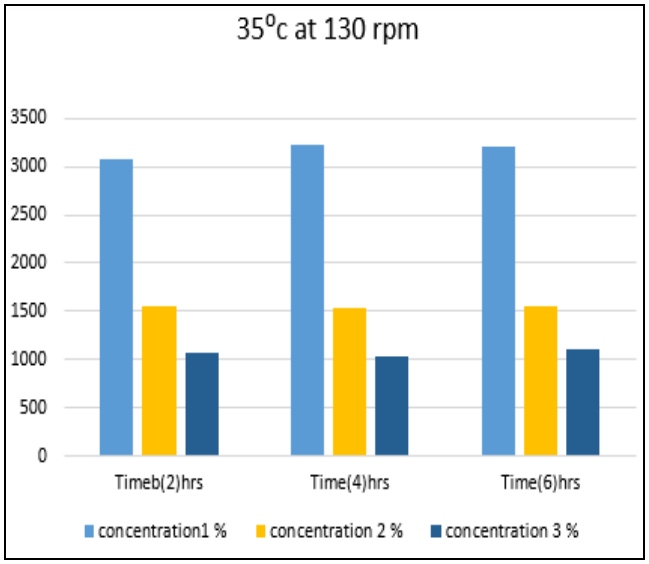

Fig 5: Total phenolic extraction at 35 ºC for 130 rpm for different concentration of peel powder

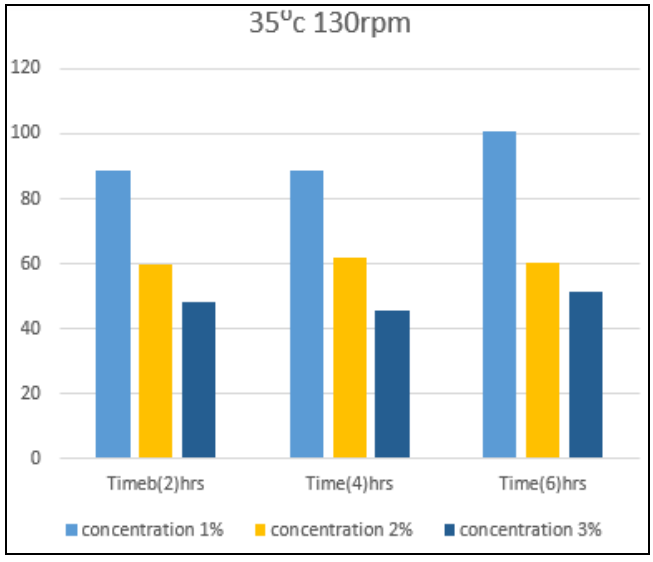

Fig 6: Total antioxidant activity at 35 ºC for 130 rpm for different concentration of peel powder

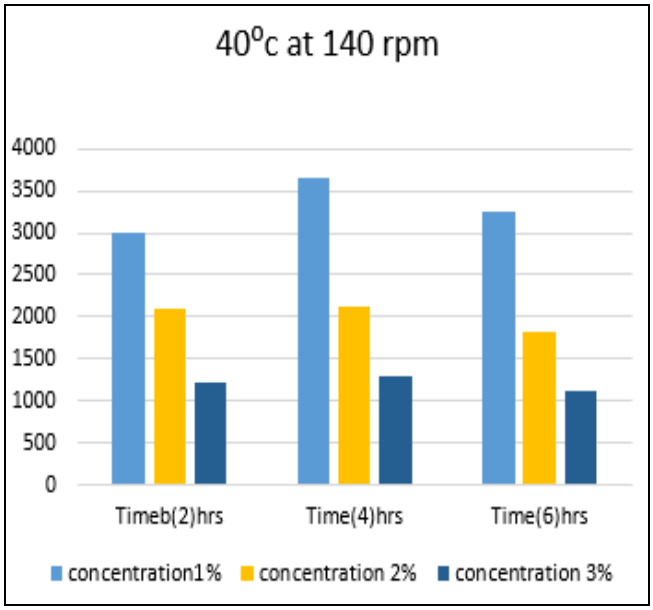

Fig 7: Total phenolic extraction at 40 ºC for 140 rpm for different concentration of peel powder

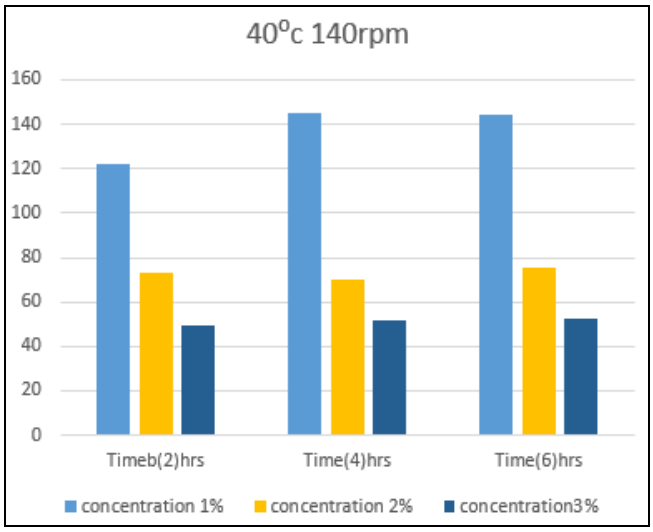

Fig 7: antioxidant activity at 40 ºC for 140 rpm for different concentration of peel powder

The effect of extraction at 37 ºC for various time interval at 125 rpm shows at 3% concentration at 6 hours there is maximum phenolic content but decrease in antioxidant activity that means positive correlation was not seen at long time interval this might be due to loss of antioxidant activity due to more extraction time. At all temperature ranges and all applied rpm for each time time interval the effect is reflected in fig 2a, 2b, 3a, 3b, 4a,4b there is positive correlation between antioxidant activity and phenolic content. The effect of increase in concentration lowers the total phenolic content extraction yield and in turn low antioxidant activity. Usually, shorter extraction time was needed with higher extraction temperature and smaller particle size (Wang, Z 2011) [23] Theoretically, under high temperatures, plant tissues are softened and the weak interactions affect the cell membranes. As a result, phenolic compounds can be easily extracted into the solvent (Sulaiman, I.S.C *et al.*, 2017) [24] prolonged extraction time decreases the extraction yield because the high temperature causes the oxidation and degradation of the desired

compounds, keeping this view the temperature ranges were selected and increase in phenolic content trend was reported with increase in temperature. Present study also throw the light on the effect of time on phenolic extraction, as time increases, there is increase in phenolic content because exposure of the sample in the solvent, allowed sufficient time for the desired compounds to migrate into the solvent. Studies have also reported, less polar compounds that could tolerate high temperatures and extraction is facilitates at high temperature. Effect of different variable that is temperature, time and peel powder concentration on antioxidant activity results of present investigation reported high yield at $40^{O}C$ and values of all 36 trials were found to be positively correlated with phenolic content. At temperature $30^{O}C$, $35^{O}C$ and $37^{O}C$ there is no significant difference in antioxidant activity was observed at all time interval but at $40^{O}C$ Increase in antioxidant activity reported till 4hrs time of extraction and which is almost not significantly different at 6 hour time interval. The effect of concentration of peel powder on antioxidant activity was found to be increased with increased concentration of peel powder but it lowers at 3% concentration this might be due to the effect of rpm on extraction of phenolic reveals that when rpm is provided increasing driving force could increase the mass transfer rate and facilitate the concentration gradient between inside and outside peel powder particle, which consequently prompted diffusion rate of phenolic antioxidants enter to the solution. Thus, present study strongly suggested that the shaking effect is the promising factor for the extraction of maximum yield of phenolic compounds from the plant material. Present study highlighted 140 rpm was optimum for phenolic extraction and antioxidant activity. (N. Azwanida, 2015) [25]

References

1. Nerín C, Tovar L, Salafranca J Behaviour. Of a new antioxidant active film versus oxidizable model compounds. J Food Eng. 2008; 84:313-320
2. Imaida K, Fukushima S, Shirai T, Ohtani M, Nakanishi K, Ito N, *et al*. Promoting activities of butylated hydroxyanisole and butylated hydroxytoluene on 2-stage urinary bladder carcinogenesis and inhibition of gamma-glutamyl trans peptidase positive foci development in the liver of rats. Carcinogenesis. 1983; 4:895-899.
3. Chen C, Pearson AM, Gray JI. Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compounds. Food Chem. 1992; 43:177-183
4. Li Y, Guo C, Yang J, Wei J, Xu J, Cheng SE, *et al*. valuation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food chemistry. 2006; 96(2): 254-260.
5. Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. Journal of Agricultural and Food chemistry. 2000; 48(10):4581-4589.
6. Kulkarni AP, Aradhya SM. Chemical changes and antioxidant activity in pomegranate arils during fruit development. Food chemistry. 2005; 93(2):319-324
7. Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (Vigna unguiculata (L.) Walp.) seed extracts. Food Chemistry. 2006; 101(1):10-19
8. Sultana B, Anwar F, Przybylski R. Antioxidant activity of phenolic components present in barks of Azadirachtaindica, Terminalia arjuna, Acacia nilotica, and Eugenia jambolana Lam. trees. Food Chemistry. 2007; 104(3):1106-1114.
9. Malviya S, Jha A, Hettiarachchy N. Antioxidant and antibacterial potential of pomegranate peel extracts. Journal of food science and technology. 2014; 51(12):4132-4137
10. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965; 16(3):144-158.
11. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry. 1996; 239(1):7076
12. Siddhuraju P, Mohan PS, Becker K. Studies on the antioxidant activity of Indian Laburnum (Cassia fistula L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. Food chemistry. 2002; 79(1):61-67
13. Kheiralla ZH, El-Gendy NS, Ahmed HA, Shaltout TH, Hussein MM. Onefactor-at-a-time (OFAT) optimization of hemicellulases production from Fusarium moniliforme in submerged fermentation. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects. 2018; 40(15):1877-1885.
14. Shiban MS, Al-Otaibi MM, Al-Zoreky NS. Antioxidant activity of pomegranate (Punicagranatum L.) fruit peels. Food and Nutrition Sciences. 2012; 3(07):991.
15. Zhang ZS, Li D, Wang LJ, Ozkan N, Chen XD, Mao ZH, *et al*. Optimization of ethanol–water extraction of lignans from flaxseed. Separation and Purification Technology. 2007; 57(1):17-24
16. Julkunen-TiittoRiitta, Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. Journal of agricultural and food chemistry. 1985; 33(2):213-217
17. Marinova EM, Yanishlieva NV. Antioxidative activity of extracts from selected species of the family Lamiaceae in sunflower oil. Food Chemistry. 1997; 58(3):245-248
18. Bajpai M, Pande A, Tewari SK, Prakash D. Phenolic contents and antioxidant activity of some food and medicinal plants. International journal of food sciences and nutrition. 2005; 56(4):287.
19. Ruanma K, Shank L, Chairote G. Phenolic content and antioxidant properties of green chilli paste and its ingredients. Maejo International Journal of Science and Technology. 2010; 4(2):193-200.
20. Singh M, Kaur M, Silakari O. Flavones An important scaffold for medicinal chemistry. European journal of medicinal chemistry. 2014; 84:206-239.
21. Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. Journal of Agricultural and Food chemistry. 2000; 48(10):4581-4589.
22. Abid M, Cheikhrouhou S, Renard CM, Bureau S, Cuvelier G, Attia H, *et al*. Characterization of pectins extracted from pomegranate peel and their gelling properties. Food chemistry. 2017; 215:318-325
23. Wang Z, Pan Z, Ma H, Atungulu GG. Extract of

phenolics from pomegranate peels. The open food science journal. 2011; 5:17-25.
24. Sulaiman ISC, Basri M, Masoumi HRF, Chee WJ, Ashari SE, Ismail M, *et al*. Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of Clinacant husnutans Lindau leaves by response surface methodology. Chemistry Central Journal. 2017; 11(1):54
25. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015; 4(196):2167-0412.