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By

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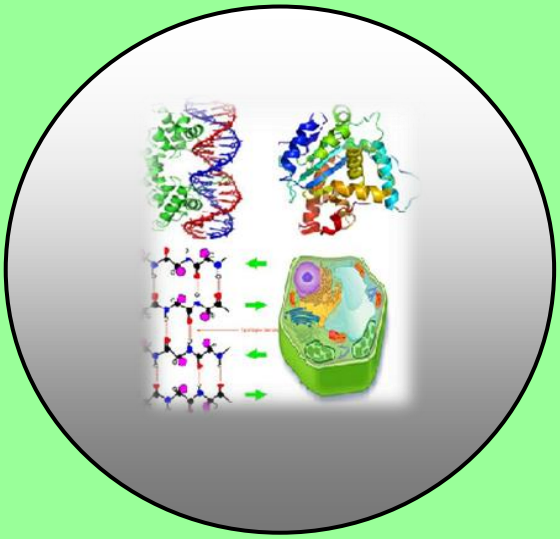

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REVIEW ARTICLE

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ABSTRACT

Antioxidant phenolic compounds are gaining popularity in the past decades for their health promoting properties. Therefore, present work is based on optimization of the extraction conditions of phenolic compounds from Flaxseed, vegetable residue Pisum peels, Aloe Vera peels by using the conventional liquid–solid method. This study focused on the effect of main independent variables on the extraction process, namely different pure solvents (ethanol, methanol, acetone, ethyl acetate, water), solvent concentration (v/v, %), number of steps for extraction, extraction temperature (°C), extraction kinetics (from 15 to180 min), pH level (2 to 10). Hence, total phenolic contents (TPC), Antioxidant activity (AA) and flavonoid content (TFC) of different fractions were determined according to the Folin-Ciocalteu, Aluminium chloride colorimetric and DPPH radical scavenging assay spectrophotometric method. The results indicated that maximum 17.59mgGAE/gTPC, 3.8mgQE/g TFC and97.47% AA obtained for Flaxseed at 70% ethanol for 120 min at 60°C and pH 4-6. Moreover, maximum 8.17mgGAE/g TPC, 1.8mgQE/g TFC, 74.22% AA obtained for Pisum peels at 90% methanol for 75min at 40°C and pH 2-4 and maximum 6.82mgGAE/g TPC, 1.07mgQE/g TFC, 75.22% AA obtained for Aloe Vera peels at 90% methanol for 60min at 60°C and pH 8. It can be concluded that one step extraction is sufficient to extract maximum phenolic content.

This investigation showed high degree correlation R^2=0.97 between total phenolic content and antioxidant activity therefore extracts might be considered as a potential source of nutraceuticals in the future.
Key Words: Total phenolic contents (TPC), Antioxidant activity (AA) and Total flavonoid content (TFC) and Solvent extraction.

INTRODUCTION

"Oxidative stress" can occur due to an imbalance between oxidants and antioxidative defense system of human body. In this condition excessively produced free radicals and reactive oxygen species (ROS) destroy different biological molecules, such as carbohydrates, proteins, lipids as well as DNA with vital molecular. These physiological damages of cells leading to numerous diseased such as carcinogenesis, mutagenesis, inflammation, DNA changes, aging and cardiovascular diseases [Dey and Kuhad, 2014]. Antioxidants substances can prevent or inhibit oxidation processes in human body and deterioration in food products [Babbar et al., 2014]. However, phenolic compounds are one of the main classes of secondary metabolites derived from phenylalanine (most plants) or tyrosine. Further, plant phenolics comprise a great diversity of compounds such as flavonoids (anthocyanins, flavanols, flavonols, flavanones) and several classes of non-flavonoids (phenolic acids, stilbenes and complex molecules derived from them [Chirons et al., 2007]. Moreover, their antioxidant potential depends on the number and arrangement of the hydroxyl groups and the extent of structural conjugation, as well as the presence of electron-donating and electron-withdrawing substituents in the ring structure [Milter and Evans, 1997]. Butylatedhydroxyanisole (BHA), Butylatedhydroxytoluene (BHT), and tert-Butylhydroquinone (TBHQ) are commonly used as synthetic antioxidants. This is due to fact that the possible role of synthetic antioxidants as promoters of carcinogenesis and liver swelling the search for endogenous protective ingredients in foods has been intensified. Antioxidants are found in various plant parts (e.g. fruits, leaves, seeds and oils)Vegetables, Flaxseed, Cereals, Berries, Wine, Tea, *Pisum*, Aloe Vera, Onion bulbs, Olive oil [Babbar et al., 2014 and Quis et al., 1996] apart from that agricultural and industrial residues are also attractive sources of natural antioxidants [Moure et al., 2007]. By-products, which remain after processing of fruit and vegetable in food processing industry, still contain anenormous amount of phenolic compounds. These antioxidant compounds from agricultural residues can increasethe stability of foods by preventing lipid peroxidation and protect cell organelles from oxidative damage [Babbar et al., 2014]. In the present work three different species are used which have good amount of antioxidants, such as Flaxseed, vegetable by product *Pisum* peels, and Aloe Vera peels.Flaxseed contains various types of phenolic compounds viz. Phenylpropanoids such as p-coumaric acid, o-coumaric acid, ferulic, p-hydroxybenzoic acid, vanillic acid, sinapic acid, secoisolariciresinol, matairesinol, enterolactone and enterodiol [Hao and Beta, 2012, Omah and Mazza, 1998]. A number ofstudies reveal that these components work well for nutritional benefit in human being [Qui et al., 1996, Kosinska et al., 2011 and Yuan et al., 2008]. *Pisum* peels (Pisumsativum) waste is also generated in large quantities whose only limited application is in the form of cattle feed [Babbar et al., 2014]. We have not yet come across any published report or literature describing the nutraceutical potential of *Pisum* peels. Generally, the seed coat (peels) plays an important role in the chemical and physical security system of the seeds which are exposed to oxidative damage, like that, UV light, oxygen, or other environmental factors.

Thus the *Pisum* seeds coat hold numerous bio-active compounds in which poly-phenols are also included, which plays a protecting role opposite to oxidative damage and may contribute to antioxidative activity [Osawa et al., 1985]. Prodelphinidin (gallocatechin, and epigallocatechin), proanthocyanidins, beta-carotene-linoleic acid , p-Coumaric acid trans, vanilic acid, ferulic acid , flavone glycosides in which apigenin-7- glucoside, apigenin-8-C-glucoside, flavonol glycosides in which quercetin-3-rutioside these are the name of antioxidants which present in *Pisum* peels(incomplete) [Troszynska et al., 2002]. Aloe Vera is mainly known for its valuable medical and therapeutic properties. Aloe Veracontains more than 200 various types of biological active compounds [Eshun and He, 2004]. Major components of Aloe Vera can be divided into five parts: phenolic components (flavonoids, anthraquinones), vitamins (B1, B2 etc.), low molecular weight components (salicylic acid, cholesterol, etc), saccharides (glucomannan, mannose, acemannan etc.) and enzymes (amylase, carboxypeptidase etc.). However, phenolic components like anthraquinones are mainly composed of aloin, aloe emodin, aloetic acid, barbaloin, emodin, anthranol, ester of cinnamic acid andbarbaloin [Ray et al., 2013]. Extraction is the first step in the isolation of phenolic compounds from plant materials. It is influenced by the chemical nature of the compounds (simple and complex phenolics) [Chirons et al., 2007]. For this purpose different conventional solvent extraction (liquid–liquid/solid–liquid) strategies have been employed. Further, extraction of phenolics from plant materials like Soxhlet extraction, maceration, microwave-assisted extraction, ultrasound-assisted extraction, high hydrostatic pressure extraction and supercritical fluid extraction etc. are used [Dey and Kuhad, 2014].Solvent extraction is a process designed to separate soluble phenolic compounds by diffusion from a solid matrix (plant tissue) using a liquid matrix (solvent).This process is widely employed for phenolic extraction from various vegetable materials [Lapornik et al., 2005, Ray et al., 2013 and Liyana and Shahidi, 2005]. A number of key factors contribute to the efficiency of the solvent extraction process: such as solvent, pH, temperature, number of extraction steps, ratio solvent/solid, and solvent/water ratio [Tan et al., 2013, Spingo et al., 2007].Numerous studies had been done to optimization of extraction process by using different source of polyphenol. On the basis of structure, physicochemical and compositions of natural sources of phenolic compounds, a universal extraction protocol is not conceivable and a definite extraction procedure must be designed and optimized for each phenolic source [Thoo et al., 2010, Silva et al., 2007, Pompeu et al., 2009].Furthermore, extraction time and temperature, solvent/solid ratio, solvent/water ratio were often investigated for different sources by Wani et al., 2015 and Dey et al., 2014.However, they have not considered a pH of antioxidants thoroughly, except the work by Chirinos et al., 2007 whereas solvent extraction is used for phenolic extraction by Silva et al., 2007. Ho et al., 2008 considered pressurized low polarity water as a solvent for extraction of polyphenol from Flaxseed, supercritical CO_2 extraction used for Aloe Vera by Bashipour et al., 2014. According to Chirinos et al., 2007, Ekvall et al., 2007 and Alberti et al., 2014 inn all the solvents such as methanol, ethanol, acetone, ethyl acetate, water and petroleum ether, maximum extraction of phenolic compounds found using methanol and ethanol. Pompeu et al., 2009 and Silva et al., 2007 reported that no significant difference arises when optimized solvent/solid (1:10to1:40 w/v) for extraction of TP Chowever Dey et al., 2014 found that the ratio of solid/ solvent influence the extraction efficiency. Ethanol and water was introduced into extraction from Flaxseed by Pag et al., 2014, Zhang et al., 2007,

and methanol and water was introduced into extraction from *Pisum* peels and for Aloe Vera by Babbar et al., 2014, Kim et al., 2014. The aim of present work is (1)Extraction of antioxidants from various sources using methanol, ethanol, acetone, ethyl acetate and water solvents to investigate the total phenolic content, total flavonoids content and antioxidant activity. (2) To determine the total phenolic content, flavonoids content and antioxidant activity for each extracted, and (3) Optimized condition for maximum extraction of total phenolic content in the sources and evaluate the effects of key factors viz. pH, time, temperature, solvent/solid, solvent strength, number of steps.

MATERIAL AND METHODS

Sample material, standards and reagents

Flaxseeds, *Pisum*, Aloe vera were purchased from local market of Jaipur. It is produced by Morarka organic foods limited (An ISO 22000:2005 certified) Rajasthan (India).Flaxseed is crushed using a coffee grinder. Peel of *Pisums* and Aloe vera were first dried in the sun for one month then crushed by grinder. Folin-Ciocalteu's phenol reagent, Butylated hydroxytoluene (BHT), Sodium Carbonate, Methanol2, 2-Diphe-nyl-1 picrylhydrazyl (DPPH) from SRL. Gallic acid, Hexane from Molychem. Aluminium chloride, Potassium acetate, Ethanol from fisher, Quercetin from Sigma Aldrich all the chemicals were AR grade.

Preliminary study of extraction conditions

The objective of the preliminary study of extraction conditions was to select the number of extraction steps and best solvent for maximum extraction. In this phenolic compound was extracted by five different solvents viz. methanol, ethanol, acetone, ethyl acetate, water at same conditions to find out the best solvents and required number of extraction stages. One gram of flax seed extracted with 10ml of methanol (solvent/material ratio 10/1) in an orbital shaker at 200 rpm in dark for 3 h at 40 ^{0}C. Then extracted mixtures were centrifuged at 2500 rpm for 1 h and supernatants were collected. The Flaxseed residues were re-extracted three times under the same conditions. The supernatants of each extracted were collected and used to determine total phenolic compounds, flavonoids content and antioxidant activities for each sample by using UV spectrophotometer. This process was repisumted again with other solvents like ethanol, acetone, ethyl acetate, water.

Optimization of the extraction procedure

Solvent/Solid ratio: After evaluation of the best solvent for maximum extraction and a number of steps in the preliminary study, now for determining optimum solvent/solid ratio took again one gram of Flaxseed as a sample extracted with20ml ethanol in an orbital shaker (company name) at 200 rpm in dark for 3 h at 40 ^{0}C. After the shaking mixture was centrifuged at 2500 rpm for 1 h at room temperature and the supernatant was collected. This process was repisumted again with 30ml & 40ml of ethanol solvent to determine total phenolic compounds, total flavonoids content, and antioxidant activities for each sample by UV spectrophotometer. Optimum solvent/solid ratio was ascertained from the results.

Solvent/Water Ratio

In this process one gram of each sample of Flaxseed was extracted with 10ml of ethanol. Different strengths of ethanol concentration evaluated were 100, 90, 70and 50 % in water for 180 min at 40 ^{0}C temperature in an orbital shaker in the dark.

After the shaking mixtures were centrifuged at 2500 rpm for 1 h at room temperature and the supernatants were collected to determine total phenolic compounds, total flavonoids content, and antioxidant activities for each sample by UV spectrophotometer. Optimum solvent/water ratio was ascertained from the results.

Extraction time

One gram of each Flaxseed was extracted with 10 ml 70% ethanol 180 min at 40 ^{0}C temperature in an orbital shaker in the dark, extraction were executed for different time periods: 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180 min,other extraction conditions were as described above.

pH evaluation

To study the effect of pH on TPC, TFC and AA. One gram of each Flaxseed was extracted with 10 ml 70% ethanol, values of pH for each sample was adjusted as 2, 4, 6, 8 10 after that samples kept for 120 min at 40 ^{0}C temperature in an orbital shaker in the dark. The pH was adjusted by using solution of 0.1M HCl and 0.1M NaOH. Subsequently. Above optimum condition was applied on these samples after the shaking mixtures were centrifuged at 2500 rpm for 1 h at room temperature and the supernatants were collected and total phenolic compounds, total flavonoids content and antioxidant activities were determined for each sample by UV spectrophotometer. Optimum pH was ascertained from the results.

Temperature evaluation:

To study the effect of temperature on TPC, TFC, AA. One gram of each Flaxseed was extracted with 10 ml 70% ethanol. Above optimum condition was applied on these samples. Samples were kept in an orbital shaker in the dark at the same condition as above only temperature were maintain at 30^{0}C, 40^{0}C, 50^{0}C, 60^{0}C. After the shaking mixtures were centrifuged at 2500 rpm for 1h at room temperature and the supernatants were collected to determine total phenolic compounds, total flavonoids content, and antioxidant activities for each sample by UV spectrophotometer. Optimum temperature was ascertained from the results.

Comparison between batch extraction and Soxhelt extraction

Maximum recovery of antioxidants was determined by soxhlet extraction. In this process first 20gm of Flaxseed, *Pisum* peel, Aloe Vera peel were taken in soxhelt with 200ml of their respective solvent viz. ethanol, methanol, and methanol. Experiment was carried out for 180min.After that samples were filtered by Whattman paper No.1 and left for evaporation of solvent after few days' dry gel were collected. Some sample of extracted were taken and Determine total phenolic compounds, total flavonoids content, and antioxidant activities for each sample by UV spectrophotometer.

Quantitative analysis

Determination of Total Polyphenol Content

The polyphenol content was quantified according to the method of (Mc. Donald et al., 2001). First of all three replicas of each extracted samples were made with different solvents. Aliquots of test samples (100 µL) were mixed with 700 µL pure methanol then added 200 µL Folin-Ciocalteau's phenol reagent (1:10 diluted with distilled water), 3 ml 10% sodium carbonate (Na_2CO_3) solution added then allowed to sit in water bath at 60^{0}C temperature for 1 minute. Now the sample is left for 15 minute prior to reading the absorbance at 740 nm. Gallic acid used as the standard for a calibration curve. Phenol content of the extracts was expressed as Gallic acid equivalent.

Determination of Total flavonoids Content (TFC)

The total flavonoids content was evaluated by the aluminum chloride colorimetric method (Chang et al., 2002) with modifications. First of all calibration standard curve was made, quercetin used as the standard for a calibration curve in which eleven point 0-62.5 ppm of different concentration of quercetin were taken and the absorbance against reagent blank has been evaluated at 415. After that first of all, three replicas of each extracted samples were made with different solvents. Aliquots of test samples (100 µL) were mixed with, .9ml methanol, after that .1ml of 10% aluminum chloride (w/v), .1ml of 1M potassium acetate (w/v) and 2.8 ml of distilled water were added. Now the sample is left for 30 minute prior to reading the absorbance at 415 nm by using UV spectrophotometer (UV-1800, Shimadzu) (Munhoz, et al., 2014)

Determination of antioxidant activity

The antioxidant activity of different extracted samples was evaluated by the method DPPH free radical communizing to the process described and modified by (Jagtap, and Bapat, 2012). Because it is easiest method, cheap, easy to understand and most important less time consuming method. The DPPH method also knows as stable organic, free radicals. This analysis is the test used to vindicate the ability of the compounds of extracts to act the giver of hydrogen atoms. In which first of all, the stock solution reagent of DPPH was prepared by dissolving 3.14 mg of DPPH in 100ml methanol (.1mM). After that first of all, three replicas of each extracted samples were made with different solvents. Aliquots of test samples (.5 mL) were mixed with 2.5ml pure methanol, after that 1ml of .1mM DPPH in methanol solution were mixed with each extract sample. The mixture was agitated vigorously and allowed to react for 30 min in darkness at room temperature. After the stand for 30 min the decrease in absorbance of the samples solution was then measured at 517 nm by using UV spectrophotometer (UV-1800, Shimadzu). A controller without extract was also calibrated and standard curve was made by using BHT (Butylated hydroxytoluene). The results were manifested as percent radical scavenging activity (% RSA). The DPPH antioxidant activity was determined by using the following formula

$$\%RSA = (A_{control} - A_{sample}) / A_{control} * 100$$

RESULTS AND ANALYSIS

Influence of different solvents on extraction of antioxidants

Different solvents were tried out for the extraction. Mostly organic solvents were used for extraction based on solubility, polarity, mass transfer characterization. The efficiency of polyphenolic extraction from Flaxseed, *Pisum* peels, and Aloe Vera peels varied in response to solvent used. From Figures 1 (A), (B), (C) we can see that ethanol and methanol extracted highest amount of total phenolic content, total flavonoids and AA. The extraction efficiency of the experimented solvents in terms of total phenolic content, total flavonoids content, antioxidant activity from the Flaxseed presented the following ordered: ethanol> methanol>acetone> ethyl acetate >water, for *Pisuml* peels methanol> water> ethanol >acetone> ethyl acetate, and for Aloe Vera peels following ordered methanol> ethanol >water> acetone> ethyl acetate. These results show that solvent has high polarity such as water and low polarity such as acetone and ethyl acetate, do not show effective extraction results. In this study ethanol and methanol were found the efficient solvents in the fulfillment of all the characteristics evaluation.

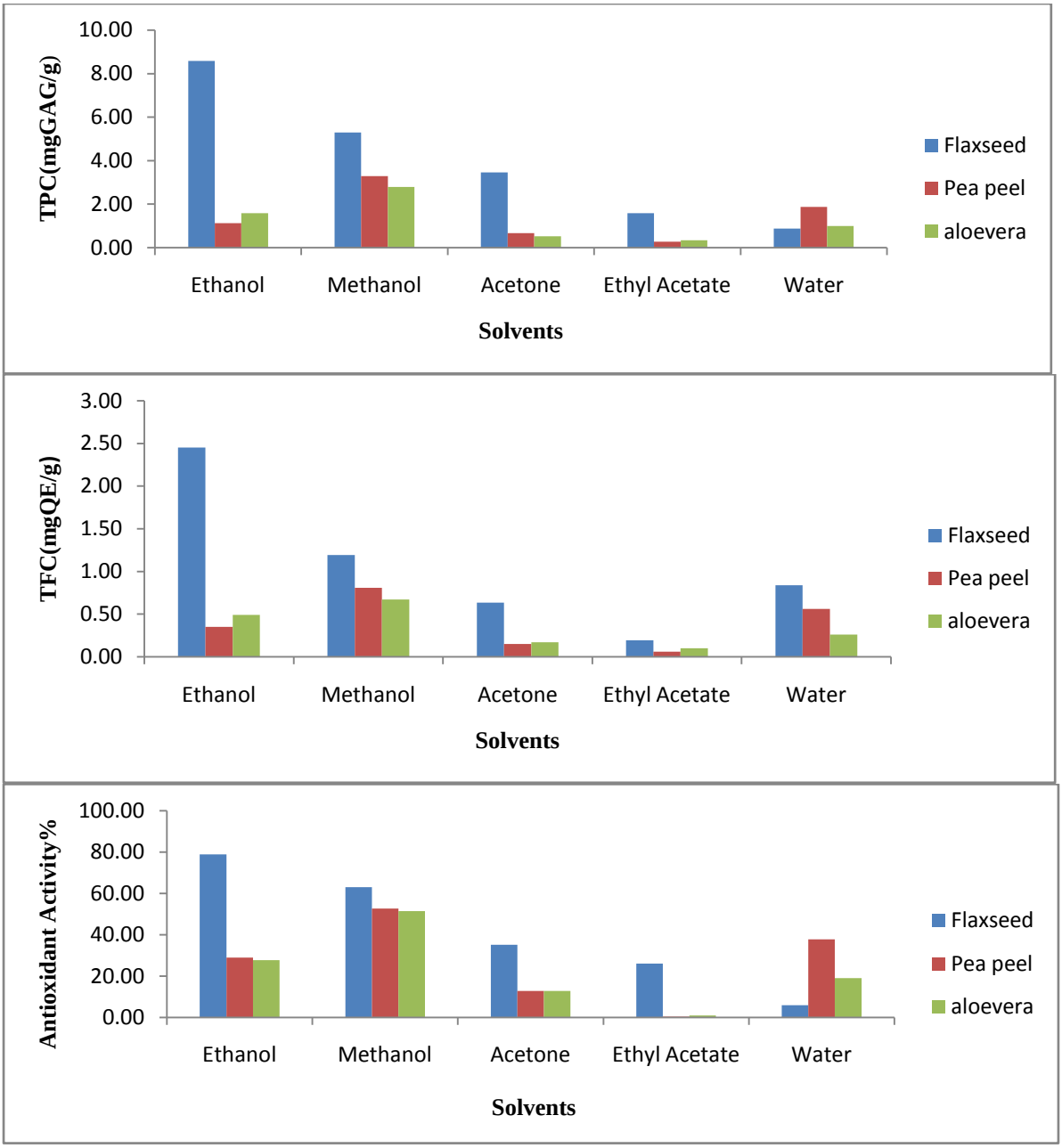


Figure 1. Effect of solvents on extraction of (A) Total phenolic content (B) Total flavonoids content (C) Antioxidant activity.

This could be because of its moderate polarity and better solubility for polyphenol compounds present in Flaxseed, *Pisum* peels, Aloe Vera peels. Flaxseed contains lignan, phenolic acids, and flavonoids as organic compound and organic compounds prefers organic solvent to dissolve Pag et al., 2014, Zhang et al., 2007. *Pisum* peels contains prodelphinidin (gallocatechin and epigallocatechin), proanthocyanidins, p-Coumaric acid trans, vanilic acid, ferulic acid , flavone glycosides in which apigenin-7- glucoside, apigenin-8-C-glucoside, flavonol glycosides in which quercetin-3-rutioside according to Babbar et al., 2014, methanol is mostly used for extraction of moderate polar antioxidant such as , p-Coumaric acid trans, vanilic acid, ferulic acid,

flavone glycosides in which apigenin-7- glucoside, apigenin-8-C-glucoside, flavonol glycosides in which quercetin-3-rutioside. Siripongvutikorn, et al., 2012, reported that methanol solvent was better for pigallocatechin, gallocatechin. Abundant phenolic compound present in Aloe Vera anthraqunonons, chromones are have moderate polarity therefore more soluble in methanol Ray et al 2013.

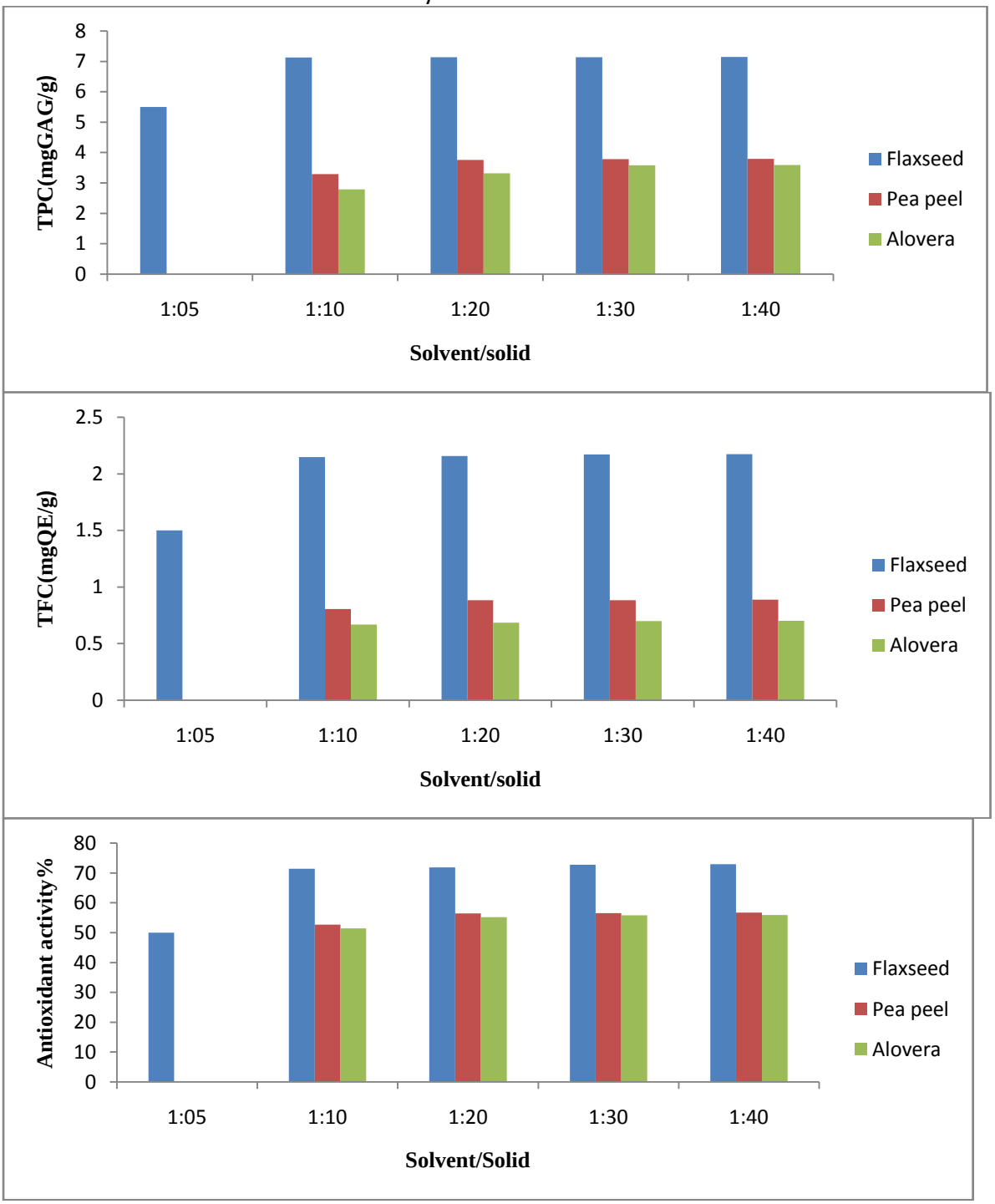


Figure 2. Effect of solvent/solid ratio on extraction of (A) Total phenolic content (B) Total flavonoids content (C) Antioxidant activity.

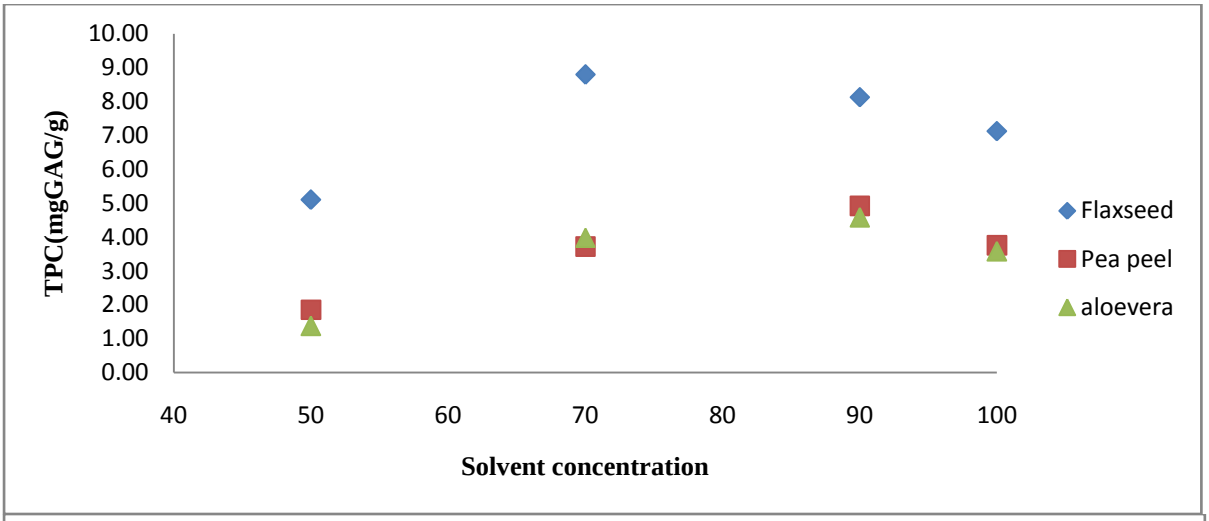
So when these compounds reached to polarity of solvents they get dissolved in solvents. Thus, total phenolic content from Flaxseed, *Pisum* peels; Aloe Vera peels present a moderately polar profile. These results are in accordance with previous study reporting Draganescu et al., 2014, Jawed et al., 2013, so for further study; we will use ethanol as solvent for Flaxseed and methanol for *Pisum* peels and Aloe Vera peels.

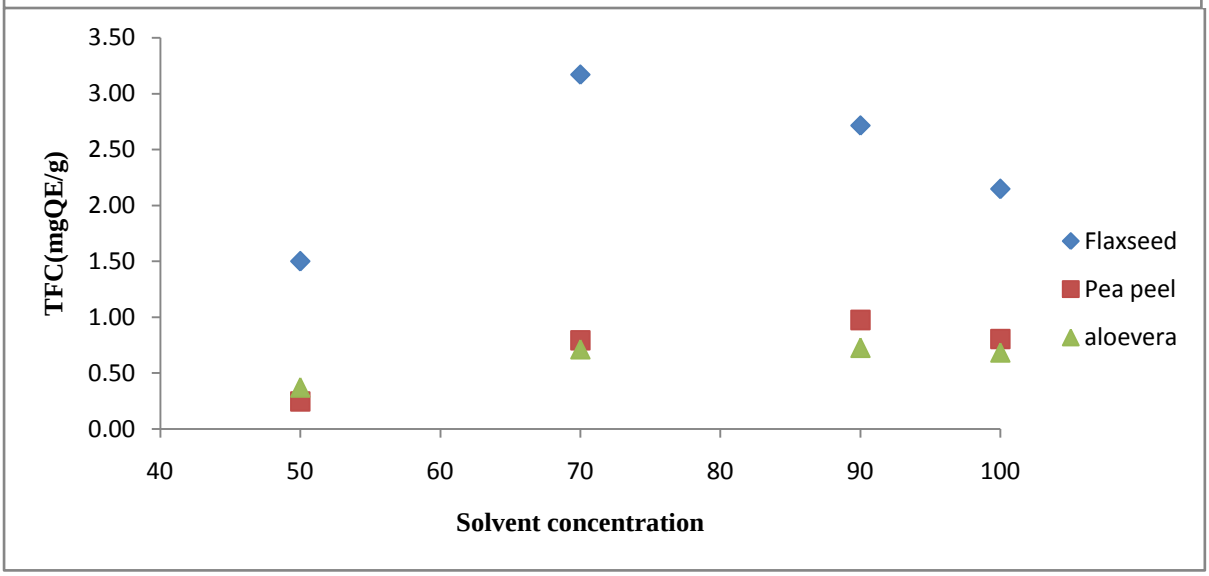
Influence of Solvent/Solid ratio on extraction of antioxidants

Solid-to-solvent ratio showed a significant effect for both TPC and DPPH scavenging property as shown in Figure.2. Among all the ratios, solid-to-solvent at 1:10 (w/v) exhibited highest amount of TPC, TFC and AA for Flaxseed, for *Pisum* peels 1:20(w/v) exhibited highest amount of TPC, TFC and AA and for Aloe Vera peels solid-to-solvent at 1:30 (w/v) exhibited highest amount of TPC, TFC and AA. These could be because more solvent gives proper mixing and better mass transfer. These results are in accordance in the literature Pompeu et al., 2009.

Influence of Solvent/water ratio on extraction of antioxidants

To maximum recovery of antioxidant from the source, we evaluated the effect of the solvent / water ratio of TPC, TFC and AA. From the result it was found that the proportion of ethanol and methanol which used for extraction in the extraction medium had a significant impact on maximum recovery of TPC, TFC and AA. From the Figure 3. It has been observed that the TFC, TPC and AA increased with the percentage increase of ethanol in water up to 70 % after that further increment of ethanol % in water the TPC, TFC and AA began to decline for Flaxseed and for *Pisum* peels, Aloe Vera increase up to 90% of methanol. The maximum amount of TPC, TFC, AA were found at 70% ethanol for Flaxseed and for *Pisum* peels, Aloe Vera the maximum amount of TPC, TFC, AA were found at 90% methanol. From these it was observed that antioxidants, like lignans molecules was polar ones, so it dissolved in water, but it is also an organic compounds so it preferred organic solvent to dissolve. Herbacetindi glucoside present in Flaxseed are dissolved effectively in ethanol Spigno, et al., 2007. Like that flavonoids are present in the form of glucoside such as herbacetin 3, 7-O-dimethyl ether, herbacetin 3, 8-O-diglucopynanoside, and kaempferol 3, 7-O-diglucopyranoside present in Pisumpeel Qiu et al., 1996 and pigallocatechin, gallocatechin present in Pisum Peels are more soluble in methanol Siripongvutikorn, et al., 2012. Like that Kim et al., 2014 reported that main components found in Aloe Vera are anthraquninons, anthrones, and chromonesare lipophilic compounds there for these preferred to dissolve in organic solvent. Aloe Vera also has partial part of flavonoids that have polar nature because of that 90% mixture of methanol/water give better extraction. It was also suggested by Pompeu et al., 2009 when the proportion of ethanol increased results in decrease of the dielectric constant of the extraction solution and a consequent decreasing in the energy needed to separate the solvent molecules, cause of that solute molecules can enter easily between solvents molecules. Silva et al., 2007[25] reported that polyphenols compounds present in a glycoside form then they high soluble in hydro alcoholic solution. These results are in accordance with previous study Babbar et al., 2014. Pure ethanol could dehydrate the vegetable cell, fruit and hence diffusion of polyphenol could be restricted. Water addition in ethanol not only increases the polarity of solvent but also decrease the cost of extraction process because ethanol is expensive solvent and mixture of water/solvent extracted better than pure solvent. More water addition was not much effective Draganescu et al., 2014. Further experiment 70% ethanol and 90% methanol were used as optimum solvent for Flaxseed, *Pisum* peels and Aloe Vera peels.





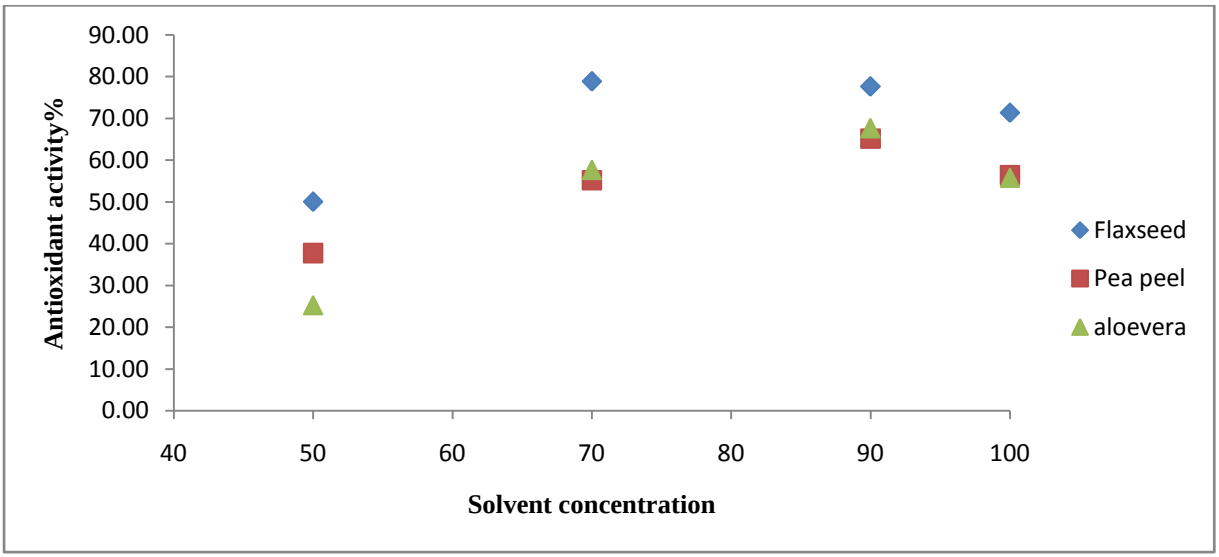


Figure 3. Effect of solvent/water ratio on extraction of (A) Total phenolic content (B) Total flavonoids content (C) Antioxidant activity

Influence of extraction kinetics on extraction of antioxidants

Extraction time is very important in minimizing cost and energy of the extraction process. From the figure 4it has been observed that for Flaxseed, *Pisum* peels and Aloe Vera 120 min, 75min and 60min respectively were selected as the optimum time with maximum value of TPC, TFC and AA. On further increase in time there is no significant effect on extraction of polyphenols. It was reported by Kim et al., 2013 that concentration of anthrons in Aloe Vera decreases with longer period because aloins are oxidized with phenolic condensation.

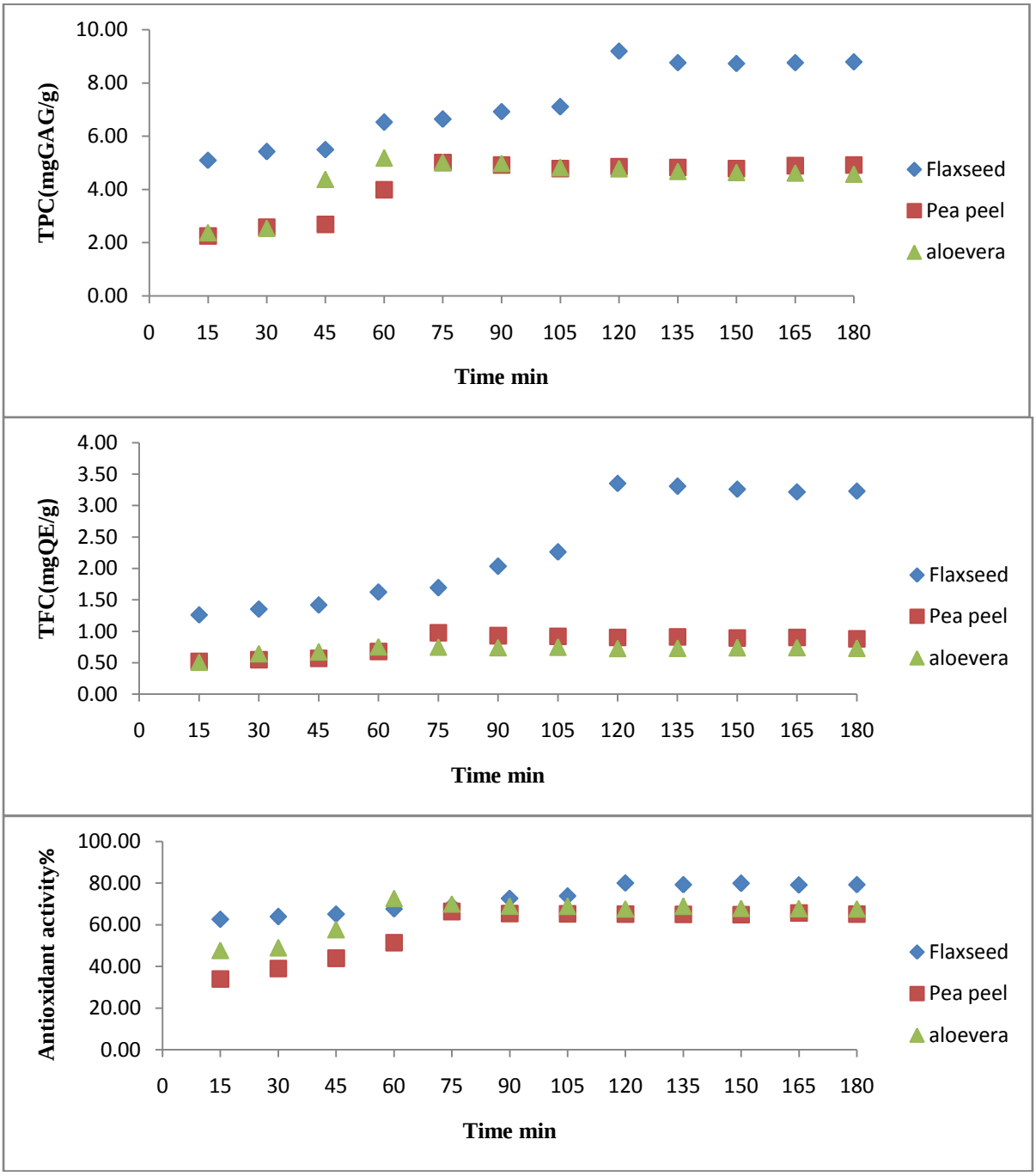
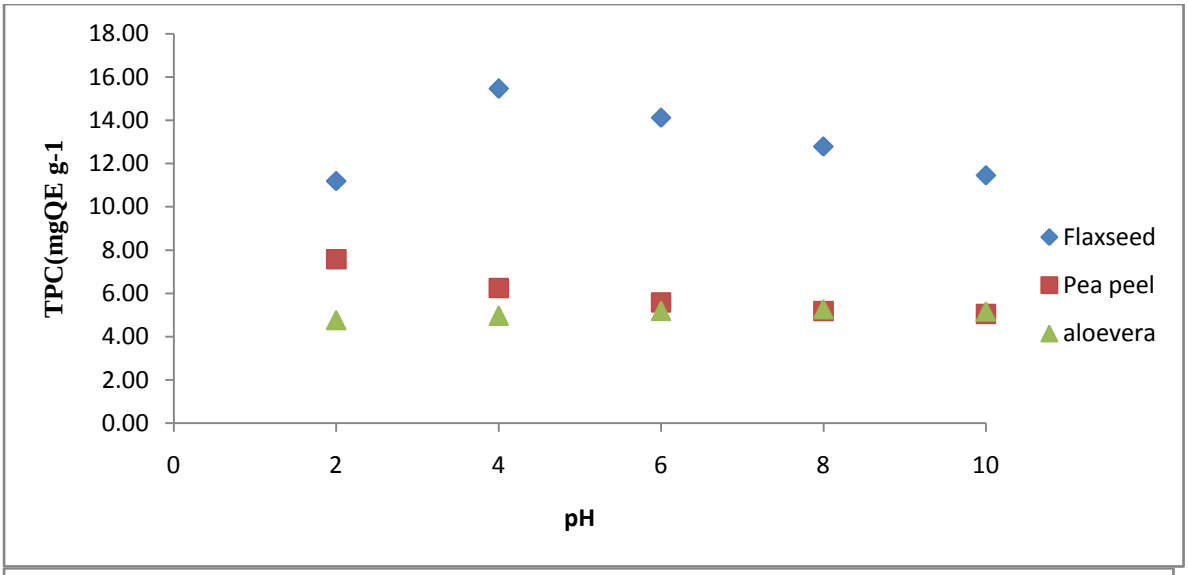
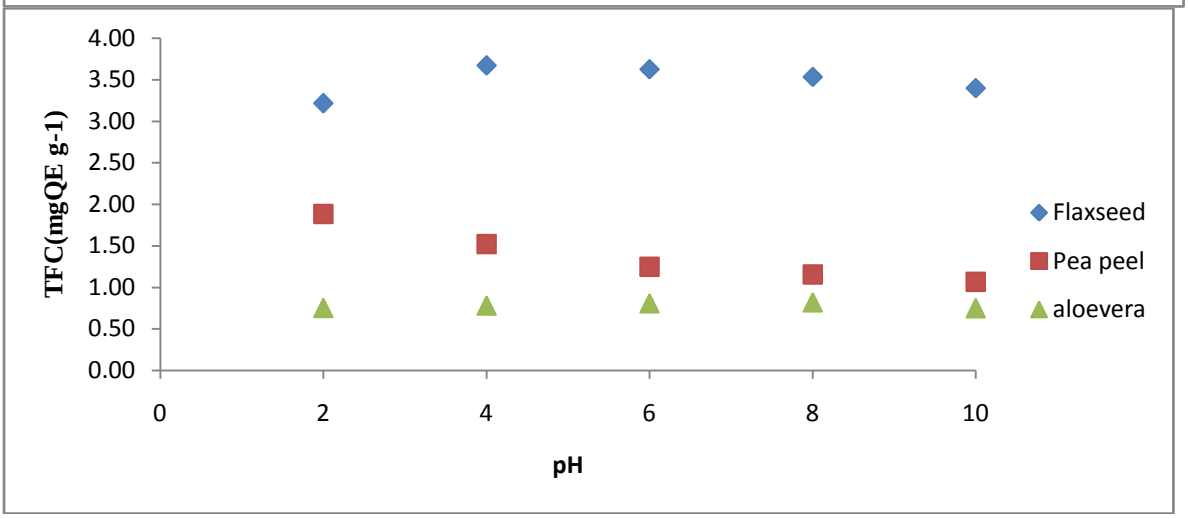


Figure 4. Effect of extraction kinetics on extraction of (A) Total phenolic content (B) Total flavonoids content (C) Antioxidant activity.





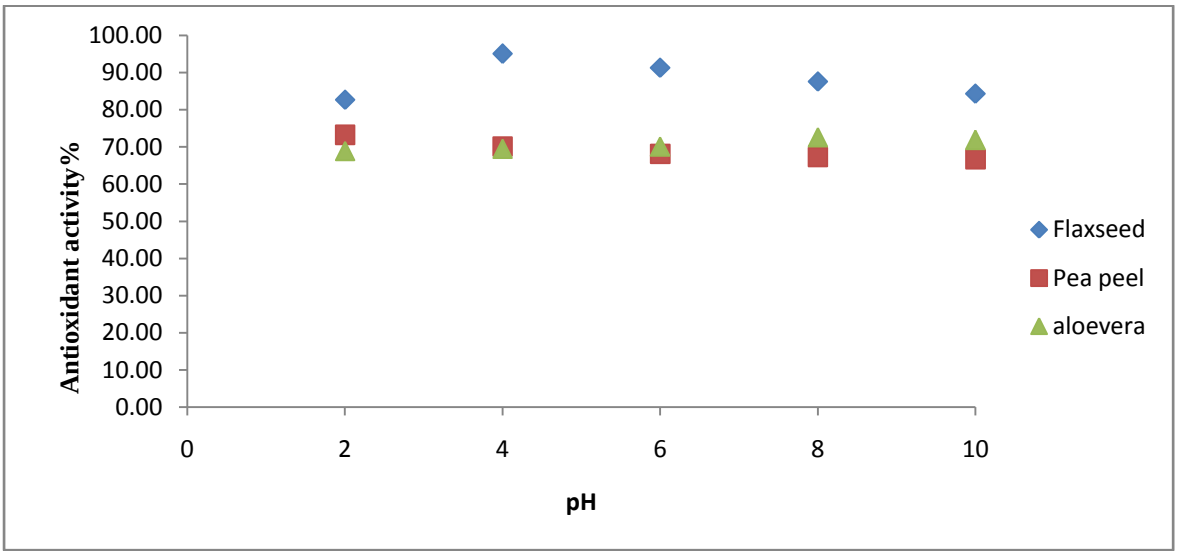


Figure 5. Effect of pH on extraction of (A) Total phenolic content (B) Total flavonoids content (C) Antioxidant activity.

Influence of pH on extraction of antioxidants

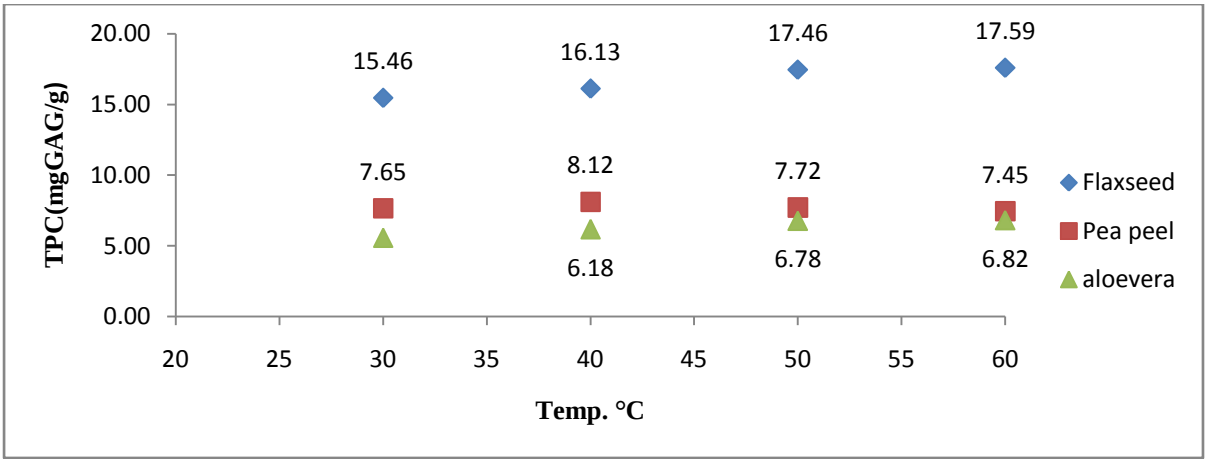
Many antioxidant polyphenol compounds in fruit vegetable and plants are usually present as a complex and covalently bound form. For that some processing methods are needed to release them so that extraction can be enhanced. From the figure 5 it has been observed that TPC, TFC and AA increase first at pH 10 after that it increase up to 4 pH then declines with decrease in pH by addition of HClin solution for Flaxseed. Ester-linked present in lignans secoisolariciresinol diglucoside (SDG) which is present in Flaxseed.

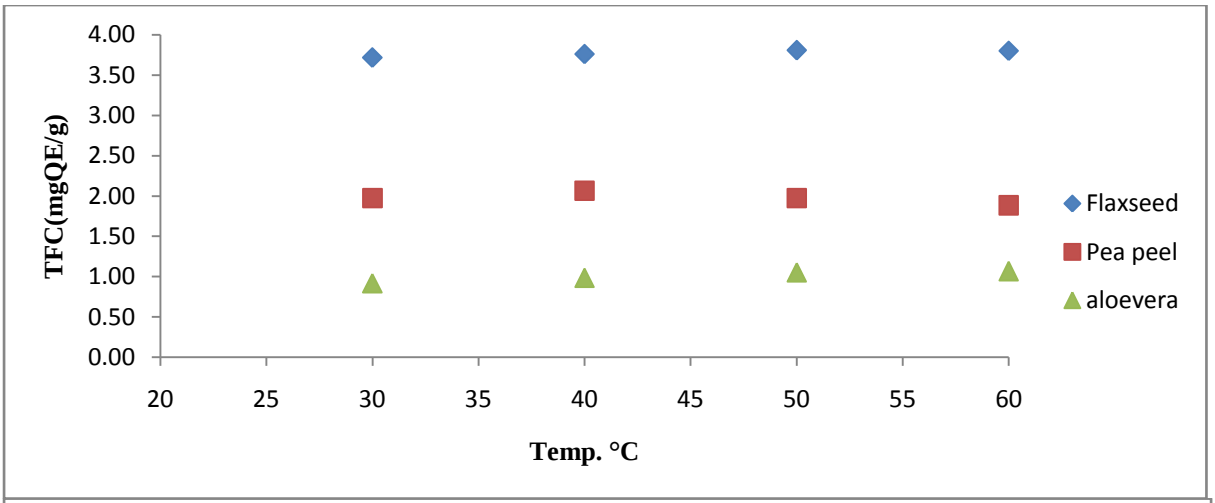
Secoisolariciresinol (SECO) is the aglycone of SDG. Lignans viz. matairesinol, isolariciresinol, pinoresinol, and lariciresinol identified in the Flaxseed and flavonoids such as 3-hydroxy-3-methylglutaric acid (HMGA) also linked with ester to SDG and other phenolic compounds such as p-coumaric acid andferulic acid glycosides also linked with SDG oligomers.

After addition of NaOH SDG, ferulic acid glucoside, p-coumaric acid glucoside, could liberated from SDG oli gomers. Ester linkages are degraded under the alkaline condition Kosińska, et al., 2011. It was suggested by Yuan, et al., 2008 that there is no need addition of alkaline in the extracted with decreasing in pH by addition HCl acid in this condition both ester-linked and glycosidic bonds are effectively break or degraded. Lignans matairesinol also stable in acidic condition. But further decreasing in pH lignans SECO start to degrade to produce anhydro-SECO. For Pisum peels it has been observed that TPC, TFC, and AA increase with decrease in pH. This could be because flavonoid such as flavone glycosides in which apigenin-7- glucoside, apigenin-8-C-glucoside, flavonol glycosides in which quercetin-3-rutioside which are present in *Pisum* peel are linked with glycoside and glycoside bonds are degraded in acidic condition. Proanthocyanidins polyphenol extracted efficiently in acidic solvent Chirinos et al., 2007 and for Aloe Vera peels TPC, TFC and AA are little bit affected by pH. This is because Chiang et al., 2012 suggested that aloin was not affected by acid or base condition and aloe-emodin was decrease in the acidic condition.

Influence of temperature on extraction of antioxidants

Effect of temperature on TPC, TFC, AA are presented in figure.6 From that it has been observed that values of TPC, TFC and AA increase with increasing temperature up to 40^0C after that it slightly begin to decline. And for Aloe Vera results shows a linear relation between TPC and temperature. This could be because of the increasing solubility of solute and diffusion of solvent and decreasing viscosity coefficient with temperature therefore solvent easily penetrates inside the complex matrix which result is in accordance with the literature Spigno et al., 2007. And cell wall bounded phenolic compounds also released by increase in temperature and dissolved in solvent. Antioxidants present in Pisum peels like flavan-3-ols pigallocatechin, gallocatechin, proanthocyanidinsare thermo sensitive Silva et al. 2007, Pompeu et al., 2009 reported that temperature is maintained to avoid degradation of these compounds to avoid degradation of these compounds must keep some certain limit of temperature. Jawed et al., 2011 reported that polyphenols present in Aloe Veralikealoin increase with increase in temperature leading to higher solubility of aloinin solvent due to which solvent penetrates easily inside the cellular matrix by this aloin molecules release faster from cellular matrix. Wang et al., 2007 who found that diffusivity of solvent and the kinetic energy increases with temperature that favored the release of bound polyphenol in a sample with the breakdown of cellular constituents of plant cells. Elevated temperature leads to increase the extraction which decreases the time to reach equilibrium.





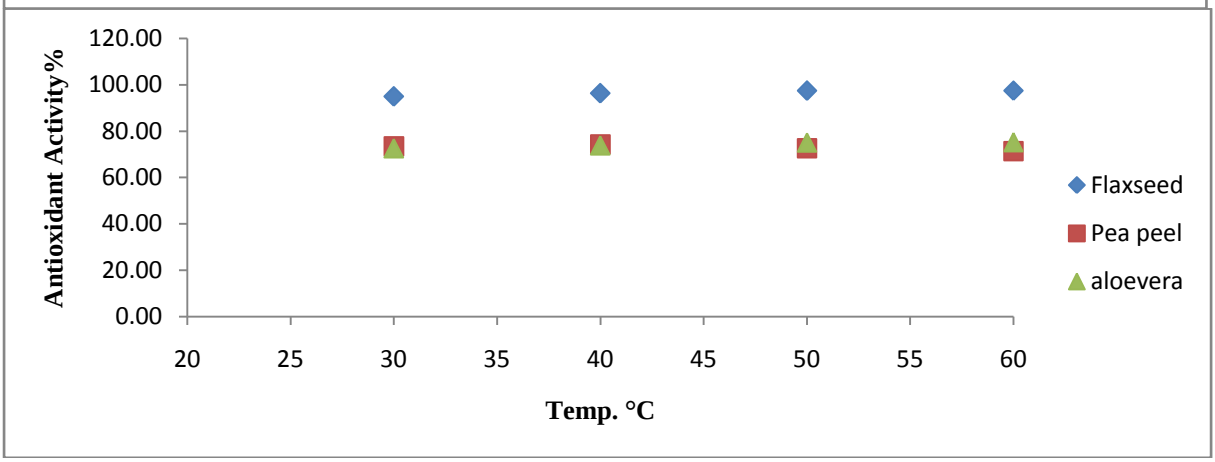


Figure 6. Effect of temperature on extraction of (A) Total phenolic content (B) Total flavonoids content (C) Antioxidant activity.

Correlation between total phenolic content and antioxidant activity and flavonoids content

To establish the correlation between total phenolic content, antioxidant activity and flavonoids content of different sources extracts, in this study a linear correlation was found between total phenolic and antioxidant activity, antioxidant activity and total flavonoids.

Among the all sources extracts analyzed in this study, value of TPC was highest for Flaxseed extract followed by *Pisum* peel and Aloe Vera peel. The vegetable residue, *Pisum* peel and Aloe Vera peel showed low value of TPC compared to Flaxseed since these were waste. Aloe Vera peel extract had low concentration of TPC and also performed lower antioxidant activity. The correlation between antioxidant activity and TPC was calculated by linear regression analysis. The value of R^2 evaluated on the basis linear correlation between AA% and TPC for extract was .97 figure7). This value showed high degree correlation between these two.

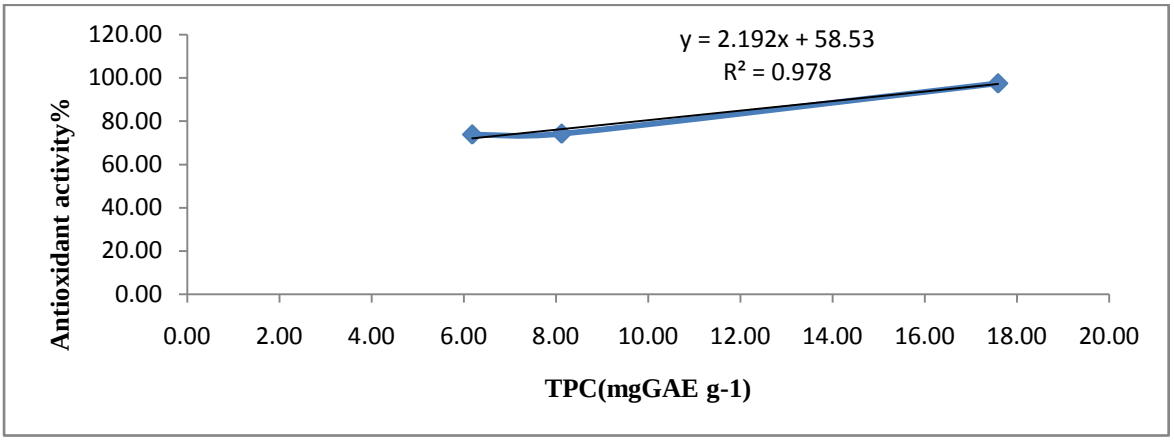


Figure 7. Correlation between total phenolic content and antioxidant activity.

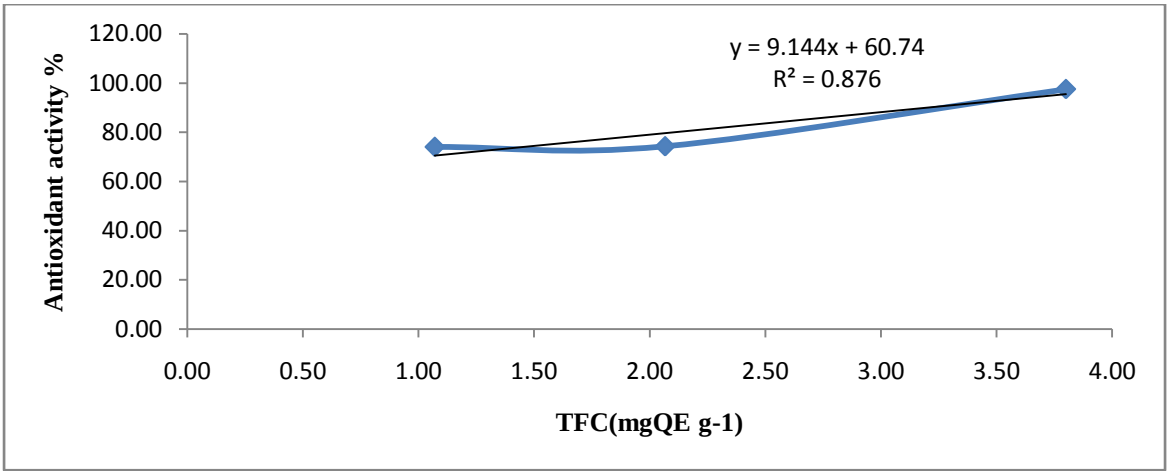


Figure 8. Correlation between total flavonoids content and antioxidant activity.

Like that the value of R^2 evaluated on the basis linear correlation between AA% and TFC for extract was .87 figure 8). This value showed lower degree correlation between antioxidant activity and flavonoids. *Pisum* peel has fewer amounts of flavonoids but performed high antioxidant activity that means *Pisum* peel has more high amounts of non-flavonoids. Babbar et al., 2014 reported high correlation between total phenolic and antioxidant activity for vegetable residue their R^2 value was .9122 by linear regression analysis. Previous study showed very high degree correlation between antioxidant activity and TPC for different food items like that cactus *Pisum* r (R^2=.97) and sorghum (R^2=.97) Rabah et al., 2004.

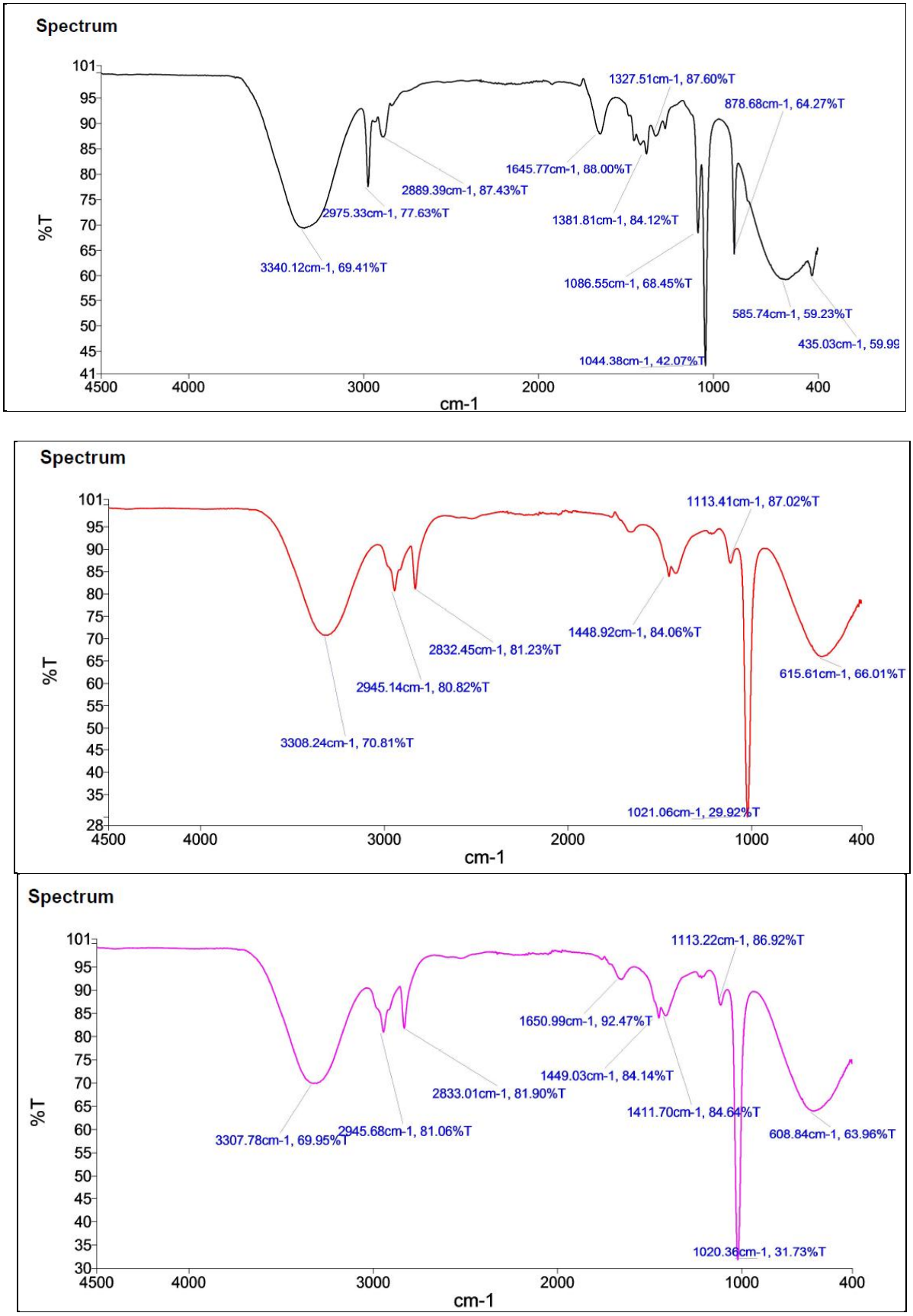


Figure 9. FT-IR profile of the Flaxseed, Pisum peel , Aloe Vera Soxhelt extracted.

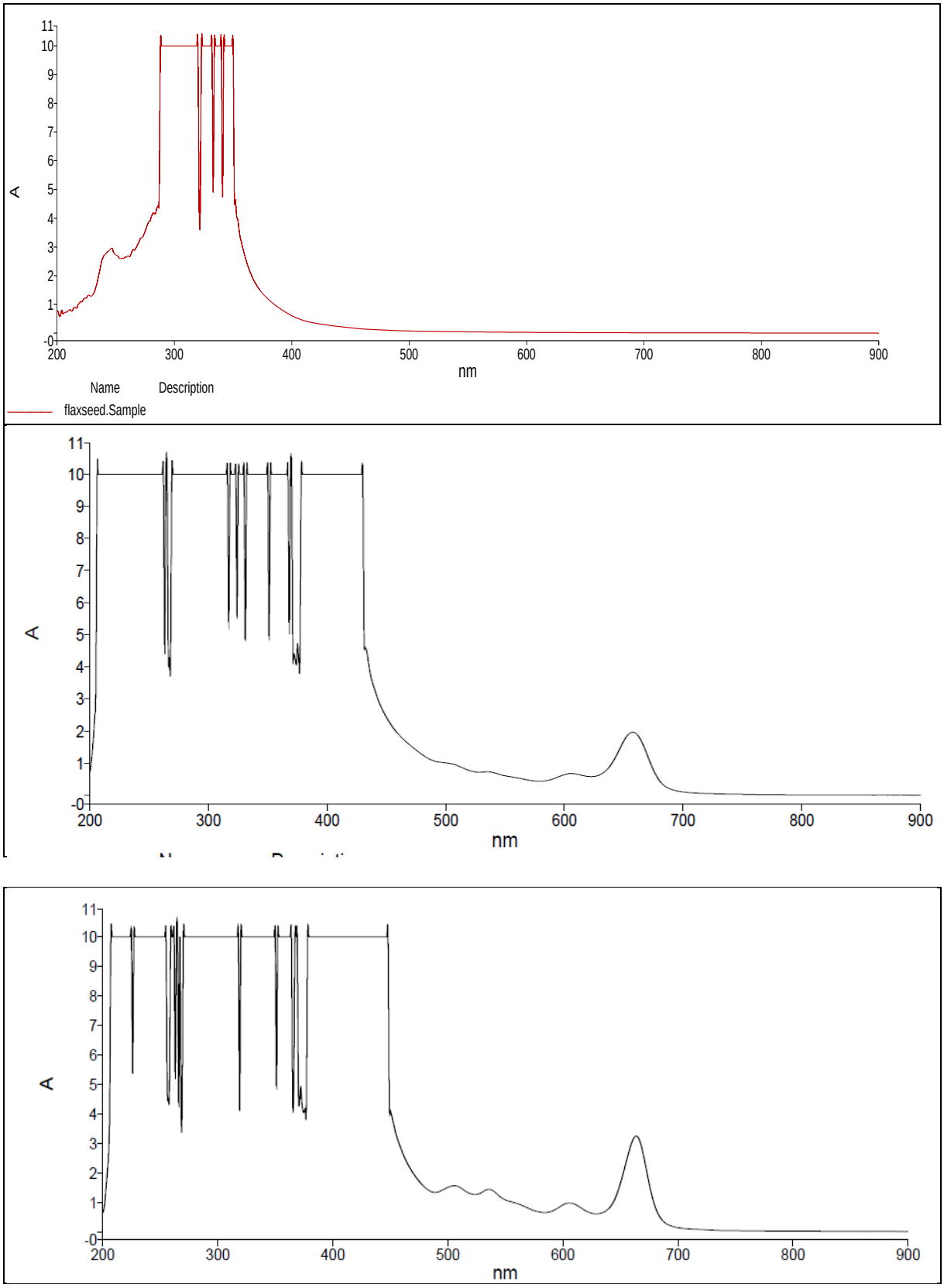


Figure 10. UV-Visible absorption profile (200–900 nm) of Flaxseed, *Pisum* peel, Aloe Vera peel showing maximum absorptions in UV region (350–700 nm).

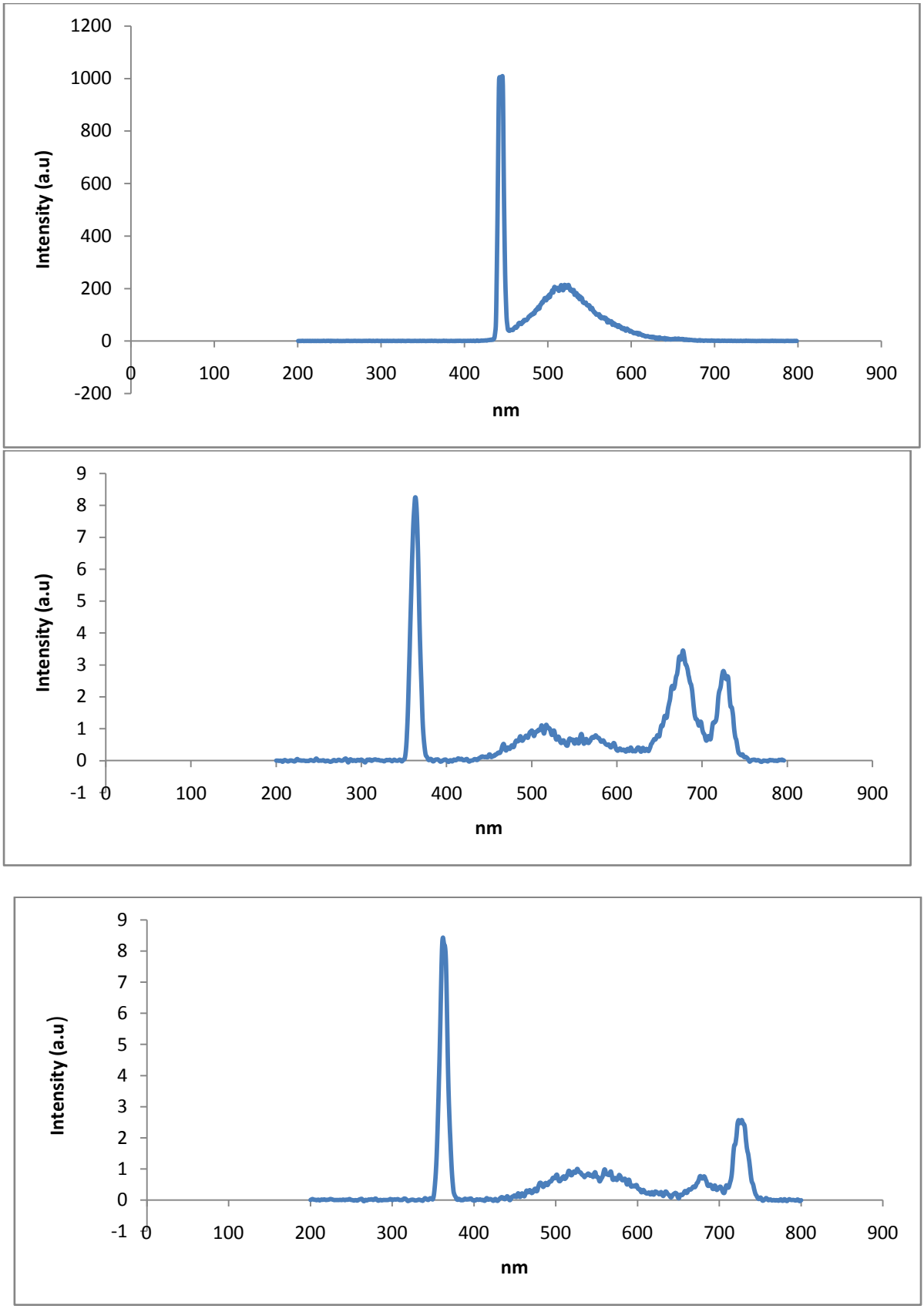


Figure 11. The Fluorescence spectral profile of Flaxseed, *Pisuml* peel, Aloe Vera peel.

The antioxidant activity of polyphenols basically shows their capability to donate hydrogen and depends mainly upon the structure of phenolic and degree of hydroxylation. We are trying to make maximum utilization of such sources as bio preservation in food product also increases use of this extracts as effective nutraceuticals.

In this study total phenolic content determined for ethanolic extraction from Flaxseed (17.59mgGAE/g) was higher than determined by Alu'datt et al., 2013 reported Total phenolic content 4.69mg/g, 68% antioxidant activity at 24°C, 1hr, pH 2, solvent/solid ratio (25/1v/w). Cameron and Hosseinian, 2013 extracted polyphenol from Flaxseed and the amount was 11.9mg/g at room temperature for 24 hrs. Extraction time was too long. Total phenolic content 4.33mg/g reported by Boussetta et al., 2013 by using high voltage discharge extraction temperature was 60 °C 25% ethanol. In *Pisum* peel total phenolic content determined for methanolic extraction (8.17mgGAE/g) was lower than determined by Babbar et al., 2014 reported (13.9mgGAE/g) but antioxidant activity was higher than 74.22% than 72%. These could because it depends upon cultivation, weather conditions. The phenolic compounds and antioxidant activity of Aloe Vera peel is not traceable in literature.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was conducted to identify the predominant functional groups present in the potent fraction. The assignments were compared with the pre-existing reports for validation purpose. FTIR profile Figure 9 indicates the presence of different polar groups such as –OH (corresponds to 3,340.10 cm^{-1}), (3,308.24 cm^{-1}) and (3,307.78 cm^{-1}) for flaxseed, pisum peel, Aloe Vera peel. Corresponds to 2,975.33, cm^{-1}), (2,945.14 cm^{-1}), (2,945.68 cm^{-1}) represent the C-H stretch for flaxseed, *pisum* peel, Aloe Vera peel. These functional groups are responsible for the polar nature of the phenolic compounds in ethanolic and methanolic extracts of Flaxseed, *Pisum* peel and Aloe Vera peel. The identification of the functional groups implies the presence of specific compounds and functional group. Its detection also helps in physico-chemical characterization of any material of interest. Strong and broad intensity of bands around 3,433 cm^{-1} was assigned to phenolic –OH stretching frequency (Chunhui et al., 2007). Very broad with high intensity band at phenolic region (at 3,433.10 cm^{-1}) indicates the accumulation of phenolic compounds in high degree. Since, total phenol contents was found high in Flaxseed, *Pisum* peel and Aloe Vera peel, the intensity of FTIR bands were also detected with high intensity. Medium intensity and broad shaped bands were observed due to =CO stretching at 1,645.77 cm^{-1}, 1,448.92 cm^{-1}, 1, 449.02 cm^{-1} indicating the presence of carbonyl compounds. Weak absorption *pisum* ks in between 700 and 610 cm^{-1} was might be due to C-H bending indicating the presence of polymerized compounds in the Flaxseed, *Pisum* peel, Aloe Vera peel.

UV spectrum

All phenolic compounds contain aromatic conjugated systems that absorb light in the UV–VIS spectral region. Figures 10 shows that phenolic compounds measured in the range of wave length (200-900nm). The maximum absorption is at around 500–800 nm for all three sources containing the phenolics group Blainski et al., 2013 reported that absorption spectra of reference compounds (gallic acid, tannic acid, catechin and pyrogallol) to be (400 to 900 nm).According to Glasl et al., 1985 each plant has a characteristic chemical composition, with uniform phenolic groups present in the same species.

Fluorescence emission

From the figure11 it has been observed that Flaxseed showed highest fluorescence emission at 460 nm (when excited at 448 nm). After that *Pisum* peel shows that fluorescence emission at 480 nm (when excited at 365 nm) and Aloe Vera peel shows that fluorescence emission at 490 nm (when excited at 365 nm). This suggests that all three sources contain highest concentration of fluoro-phore compounds presumably, flavonoids, flavonols and anthraquinone, lignin, moiety.

CONCLUSION

The experimental studies approach succeeded in optimizing the extraction conditions of the phenolic antioxidants from Flaxseed, *Pisum* peels and Aloe Vera peels using solvent extraction method. The effect of main independent variables, namely different pure solvents (ethanol, methanol, acetone, ethyl acetate, water), solvent concentration (50, 70, 90,100 v/v, %), number of steps for extraction, extraction temperature (^{o}C), extraction kinetics (from 15 to180 min), pH (2 to 10) on total phenolic content, total flavonoids content and antioxidant activity. Total phenolic contents (TPC), Antioxidant activity (AA) and Total flavonoid content (TFC) of different fractions were determined according to the Folin-Ciocalteu, Aluminum chloride colorimetric and DPPH radical scavenging assay spectrophotometric method. The results indicated that maximum 17.59 mgGAE/g TPC, 3.8 mgQE/g TFC and 97.47% AA obtained for Flaxseed at 70% ethanol for 120 min at 60°C and pH 4-6. Moreover, maximum 8.17mgGAE/g TPC, 1.8mgQE/g TFC, 74.22% AA obtained for Pisum peels at 90% methanol for 75min at 40°C and pH 2-4 and maximum 6.82 mgGAE/g TPC, 1.07 mgQE/g TFC, 75.22% AA obtained for Aloe Vera peels at 90% methanol for 60min at 60°C and pH 8. It can be concluded that one step extraction is sufficient to extract maximum phenolic content. A further investigation is needed for the isolation and identification of the active components and to elucidate its mechanisms of action as well as their potential in biological activity and antioxidant activity. This investigation showed high degree correlation R^2=0.97 between total phenolic content and antioxidant activity therefore extracts might be considered as a potential source of nutraceuticals in the future.

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