



# Plant Archives

Journal home page: [www.plantarchives.org](http://www.plantarchives.org)

DOI Url: <https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.035>

## EFFECT OF MATURITY STAGES AND SOLVENT POLARITY ON PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF GUAVA CULTIVARS

Surabhi Shukla, Radha Kushwaha, Monika Singh, Jyotsana Singh, Vinita Puranik and Devinder Kaur\*

Centre of Food Technology, University of Allahabad, Allahabad, 211002, India

\*E-mail: [devi\\_sonu@yahoo.com](mailto:devi_sonu@yahoo.com)

(Date of Receiving-29-09-2020; Date of Acceptance-23-12-2020)

### ABSTRACT

Tropical fruits claim to have phenolic compounds that have been reported to possess strong antioxidant activity. The study investigated antioxidant activity and total phenolic content of five Guava cultivars namely Allahabad Safeda, Allahabad Surkha, Lalit, Chittidar and Apple color harvested at three fruit ripening stages (unripe, semi-ripe and ripe) and extracted by four solvents (ethanol, methanol, acetone and water) with different polarities (50%, 70% and 100%). The findings suggest that guava cultivars with maturity stages and extracting solvent affected its phenolic content and antioxidant activity significantly. Pure solvents were inefficient extraction medium for antioxidants. Enhanced extraction yields were obtained from solvent containing higher water concentrations and 50% ethanol is a recommended solvent for extracting antioxidants compounds from guava fruit. High correlations between phenolic compositions and antioxidant activities of guava extracts were observed. The total phenolics and antioxidant capacity decreases as the ripening proceeds (unripe>semi-ripe>ripe) in all five cultivars. This study facilitates information on the total phenolic content and antioxidant capacities of guava cultivars which is an important commercial fruit. Results obtained showed that solvent polarities and ripening stages played the major role in extraction of polyphenolics from Guava. The significant declines in the levels of total phenolics and antioxidant capacity during fruit development suggest that the unripe fruits can also be taken for processing into nutraceutical foods.

**Keywords:** Guava, solvent polarity, maturity stages, antioxidant activity, cultivars.

### INTRODUCTION

The use of plants as antioxidants in processed foods is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants. There is a growing interest in studies of plant extracts and essential oils for their potential antioxidant activity. Fruit antioxidants had synergistic effects and protective properties against various degenerative disorders. The protection mechanism generally functions at several different levels within cells in human body by inhibiting the formation of free radical species, intercepting radical-chained reactions, converting existing free radicals into less harmful molecules and repairing oxidative damage (Du *et al.*, 2009). Guava (*Psidium guajava*) is one of the most important fruit crop in tropical and subtropical regions of the world and claims superiority over different fruits by virtue of its commercial and nutritional values. Guava cultivars display a great diversity in tree size, bearing habit, and yield, as well as in fruit size, shape, quality and other physico-chemical characteristics. The fruit has a sweet taste with a little astringency. The astringency may be assigned to the polyphenols present in it and immature fruits are used in local medicine to treat gastroenteritis, diarrhea and dysentery (Ojewole *et al.*, 2008). According to Misra and Seshadri (1968) phenolic compounds such as myricetin, apigenin, ellagic acid and anthocyanins are also present at high levels in guava fruits. Thus guava is at top among tropical fruits when it comes to disease fighting antioxidants. Efforts have been undertaken

to extract antioxidant from plant sources as extraction is the initial step in the isolation of bioactive components from plant materials. The aim of an extraction process is to obtain the maximum concentration of target compounds and of the highest antioxidant activity of the extracts (Spigno *et al.*, 2007). Extraction is influenced by the chemical nature of the compounds, the extraction technique employed, and the presence of interfering substances (Chirinos *et al.*, 2007). The solvent extraction has been widely used to extract bioactive components from plants. Solvent extraction is a process designed to separate soluble antioxidant compounds by diffusion from a solid matrix (plant tissue) using a liquid matrix (solvent). It is noted that a solvent system for extraction is selected according to the purpose of extraction such as preparation or analysis, the nature of interested components, the physicochemical properties of the matrix, the availability of reagents and equipments, cost, and safety concerns (Yu *et al.*, 2005). The commonly used solvents for extracting antioxidant were methanol, ethanol, and acetone either singly or in combination with aqueous (Lim *et al.*, 2007; Thaipong *et al.*, 2006; Tachakittirungrod *et al.*, 2007; Kahkonen *et al.*, 1999; Velioglu *et al.*, 1998; Zielinski and Kozłowska 2000). The polarities of the different organic solvent greatly influence the selection of a specific solvent for the extraction of a specific group of bioactive compounds. Extraction of antioxidant is influenced by the extraction technique employed (Chirinos *et al.*, 2007). Various techniques have been applied to extract antioxidants from plant materials and other foodstuffs. The objectives

of this research were to investigate the effect of different extraction solvents on the phenolic and antioxidant content of guava fruit varying with its maturity and cultivars.

## MATERIALS AND METHODS

**Sample-** Guava cultivars were collected at different ripening stages from an orchard in Khusroo bagh (under the Department of Horticulture and Food Processing, Uttar Pradesh Government), Allahabad and stored at  $15\pm 2^\circ\text{C}$  with relative humidity of 90-95%.

**Chemicals-** Ascorbic acid, quercetin, gallic acid standards were obtained from Sigma Aldrich Chemical Co. St. Louis, Missouri (USA). The entire chemical used for analysis were of analytical grade and procured from Sisco Research Laboratories (SRL) Pvt Ltd, Mumbai, India; Thomas Baker (chemical) Ltd, Mumbai, India; Qualigens Fine Chemicals, Mumbai, India; CDH Pvt, Ltd, New Delhi, India; Spectrochem Pvt Ltd, Mumbai.

**Sample preparation-** Fresh fruits (2.0 g) were extracted with 5 ml solvent of different polarities, aqueous ethanol (ethanol: water; 70:30;50:50 v/v), methanol (methanol: water, 70:30;50:50 v/v), acetone (acetone: water70:30;50:50v/v) and absolute ethanol, methanol, acetone, water for 24 hours at room temperature in orbital shaker (REMI,C1S-24BL).The extracts were separated from the residues by filtering through Whatmann No.1 filter paper. The residues were extracted twice with the fresh solvent and extracts were combined. The extracts were stored in a refrigerator ( $5\pm 2^\circ\text{C}$ ) until used for further analysis.

**Total phenolic content-** Total phenolic content was determined by the Folin Ciocalteu method using gallic acid monohydrate as standard. It was dissolved in various extraction solvents. An aliquot (0.05 mL) of sample or standard was placed in test tube and the volume was adjusted to 6 ml with deionised water. Then 0.3 mL of Folin Ciocalteu was added to all tubes. After 8 minutes 0.9 ml of 20% sodium carbonate was added to the mixture and then incubated for 30 minutes at  $40^\circ\text{C}$ . Absorbance of the resultant blue colour was measured at 765 nm in spectrophotometer (Model: Evolution 600, Thermo scientific, US). Total phenolics were expressed as mg gallic acid equivalent/gm weight.

**DPPH radical scavenging activity-** Free radical scavenging activity of extracts was measured by the slightly modified method of Alothman *et al.*, 2009The antioxidant capacity of the fruit extracts was studied through the evaluation of the free radical scavenging effect on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. An aliquot (100  $\mu\text{L}$ ) of fruit extract was mixed with 3.9 ml of 0.1 mM DPPH methanolic solution. The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. The absorbance was measured at 515 nm. Results were expressed as percentage of inhibition of the DPPH radical. Ascorbic acid was used as standard.

**Free radical scavenging activity using ABTS-** A modified procedure using ABTS (2,2-azino-di-(3-

ethylbenzothiazolone-sulphonic acid) as described by Re *et al.*, 1999 was used. The ABTS+ stock solution (7 mM) was prepared through reaction of 7 mM ABTS and 2.45 mM of potassium persulphate as the oxidizing agent. The working solution of ABTS+ was obtained by diluting the stock solution in ethanol to give an absorption of  $0.70\pm 0.02$  at  $k = 734\text{ nm}$ . Sample extracts (10  $\mu\text{L}$ ) were added to 90  $\mu\text{L}$  of ABTS+ solution and absorbance readings at 734 nm were taken at  $30^\circ\text{C}$  exactly 10 min after initial mixing. The percentage inhibition of ABTS+ of the test sample and known solutions of Trolox was calculated by the following formula: %inhibition =  $100 \cdot (A_0 - A)/A_0$  where  $A_0$  was the beginning absorbance at 734 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the test sample at 734 nm. The calibration curve between %inhibition and known solutions of Trolox (100–2000  $\mu\text{M}$ ) was then established. The radical-scavenging activity of the test samples was expressed as Trolox equivalent antioxidant capacity (TEAC  $\mu\text{molTrolox/g}$ ).

**Statistical analysis:** The tests were performed in triplicate, and the values were presented as means  $\pm$  the standard deviations. Analysis of variance (ANOVA) and Duncan's method were carried out to relate significant differences between samples and solvents at the 95% level of confidence, using the SPSS Statistics 20 System for windows (SPSS Statistical Software, Inc., Chicago, IL, USA) software package.

## RESULT & DISCUSSION

**Extraction of Antioxidants from Guava-** Fruits and vegetable contain variety of compounds showing antioxidant properties. Different methods have been developed to determine the antioxidant property of different plant samples (Guo *et al.*, 2003). The recovery of the compounds in solvent dependent on the solubility of the antioxidant compounds in solvents used for extraction. Thus, the polarity of solvents can increase the solubility of the antioxidant compounds (Alothman *et al.*, 2009). In this study, four different methods have been used for the evaluation of the antioxidant capacity of the guava extracts namely ferric reducing antioxidant power assay (FRAP assay), DPPH free radical scavenging assay, reducing Power and ABTS+ radical scavenging capacity.

**Total Polyphenolics-** The polyphenolic compounds in plant extracts are more often associated with other molecules like protein, polysaccharides, terpenes, chlorophyll and inorganic compounds. Hence, it requires suitable solvent for maximum extraction of polyphenols (Michalak *et al.*, 2017). In the present study different solvent extracts used for extraction of total phenolics and differences in values were observed with increased in the polarity. The TPC values were in the following the order (from high to low): 50% ethanol > 50% methanol > 50% acetone > 70% ethanol > 70% methanol > 70% acetone > 100% ethanol > 100% methanol > 100% acetone > water for all selected

guava cultivars.

The values of total phenolics of Guava cultivars in various solvents extracts varied from 10.23 to 70.67 GAE mg/g. The highest total phenolic was obtained with 50% ethanol at unripe stage followed by semi-ripe and ripe stages in all the cultivars taken for the study (Figure 1). The difference in the extract yield from guava might be due to different availability of extractable components resulting from the varied chemical composition of the fruit. The amount of the antioxidant components that can be extracted is mainly affected by the vigour of the extraction procedure, ripening stages and cultivars which probably may vary from sample to sample. This might be due to the fact that phenolics are often extracted in more polar solvents such as aqueous ethanol, acetone and methanol. Among the cultivars, the cultivar Red guava had the maximum amount (70.67 GAE mg/g) of phenolics as compare to cultivar Lalit (62.59 GAE mg/g), Surkha (59.34), Safeda (58.12), and Chittidar (57.88). The results of the total phenolics of selected fruits in this study are in agreement Thaipong *et al.*, (2006), and they reported a concentration of total phenolics 34.49 (GAE mg/g) in Allahabad safedaguava which is lower than present results.

Musa *et al.*, (2011) found that pure solvents were inefficient extraction media for antioxidant. Luximon and Ramma *et al.*, (2003) reported that white pulp guavas had higher ascorbic acid content and total phenolics than the pink pulp. The total phenolics were 24.73 and 12.64 GAE mg/g in white and pink pulp respectively. Patthamakanoporn *et al.*, (2008) reported 14.80 GAE mg/g total phenolics in guava. The polyphenolics content of strawberry guavas and common guavas were 44.39 and 19.52 GAE mg/g, respectively (McCook-Russell *et al.*, 2012). Alothman *et al.*, (2009) reported phenolics in range 12.30 to 19.10 GAE mg/g in guava with maximum extraction observed in 90 % acetone followed by 90% ethanol with no significant difference between them ( $p < 0.05$ ). Ethanol and water mixtures are commonly used for the extraction of phenols from plant materials (Patthamakanokporn *et al.*, 2008; McCook Russell *et al.*, 2012; Bahorun *et al.*, 2004). This is due to the wide range of phenols that the aqueous ethanol mixtures can dissolve. Total phenol of Allahabad safeda and Bhavanagar varieties of guava was assessed by Viraj and Pillai, (2012) the results revealed that phenolics was higher in Bhavnagar red variety compared to Allahabad safeda. Gokmen (2009) also suggested ethanol:water (50:50) as most appropriate solvent, based on results attained after performing thousands of food samples. Wide range of total phenolics has been reported by various authors in gooseberry which might be due to the difference in climacteric conditions, raw material composition and used solvents of different polarities for analysis (Prasad *et al.*, 2009). Therefore, the selection of an appropriate solvent system is one of the most relevant steps in optimizing the recovery of total phenolic content and other antioxidant compounds from a sample (Gokmen

*et al.*, 2009).

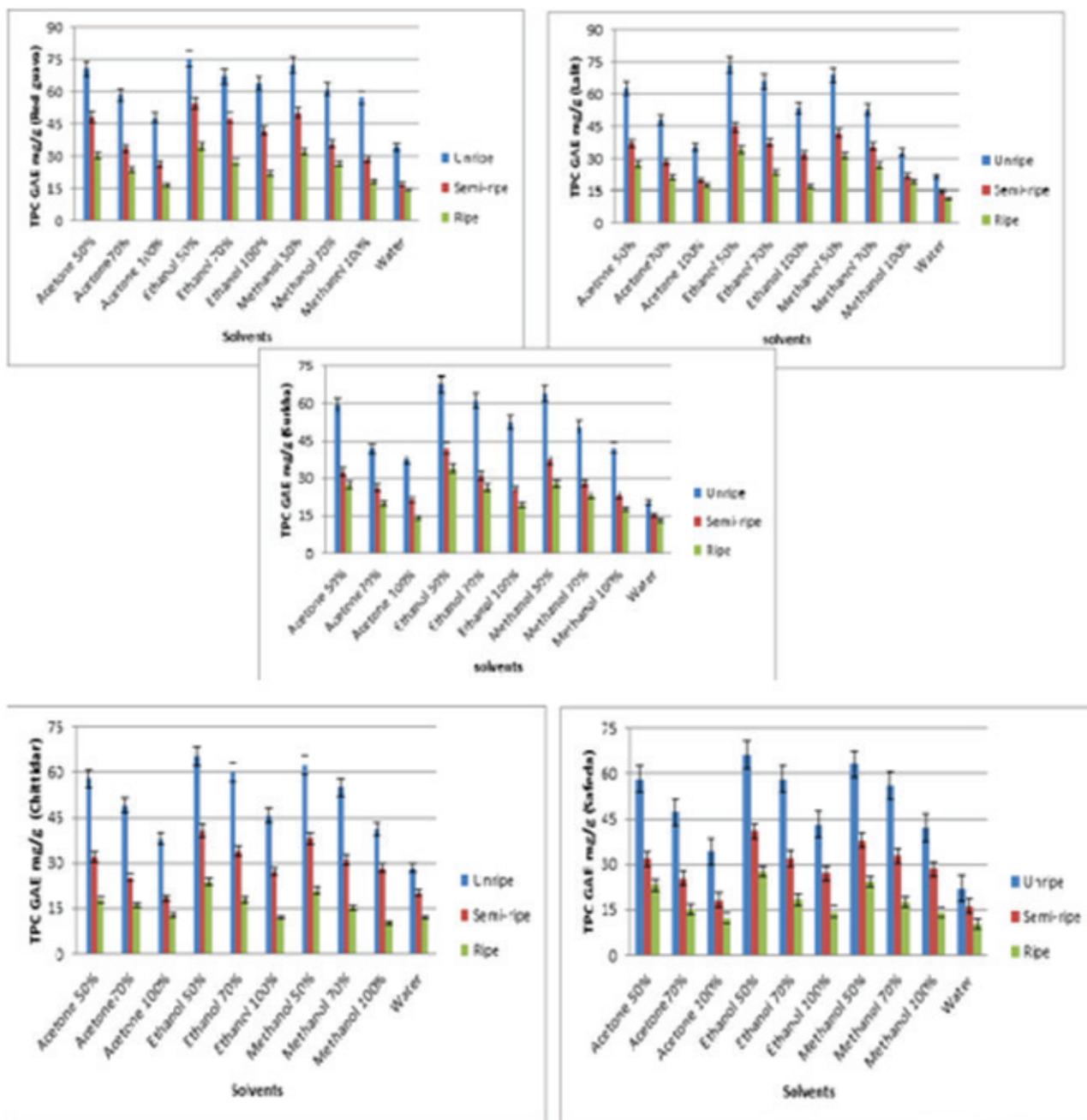
**Total Flavanoid Content (TFC)-** Flavonoids are one of the important secondary metabolites of plant which include flavones, flavanols and condensed tannins. Various studies suggest that the consumption of flavonoid-rich foods protects human against diseases associated with oxidative stress (Liu *et al.*, 2008). In order to estimate the potential effect of solvent on the amount of TFC, the guava fruit was subjected to different solvent for the extraction of flavanoids. The range of TFC in fruit from all the cultivars at different solvents was ranged from 5.02 to 22.04 mg QCE/g for unripe fruit, from 3.21 to 20.39 mg QCE/g for semiripe fruit, and 2.78 to 12.98 mg QCE/g for ripe fruit. The TFC values of the extracts are presented in Figure 2, for different cultivars i.e., Red guava, Lalit, Surkha, Chittidar, and Safeda, respectively. The TFC values were highest for Safeda followed by Lalit, Chittidar, Surkha, and Red guava. Results showed that 50% ethanol was the best medium for the extraction of flavonoid content. The TFC value of guava in different solvents ranges from 2.78 to 22.04 mg QCE/g in decreasing order of 50 % ethanol > 50% methanol > 50% acetone > 70% ethanol > 70% methanol > 70% acetone > 100% ethanol > 100% methanol > 100% acetone > water.

Phenolic acids and flavonoids have been reported to be the main phytochemicals responsible for the antioxidant capacity of fruit and vegetables. Plant-derived polyphenols display characteristic inhibitory patterns towards the oxidative reaction *in vitro* and *in vivo* models. Verma *et al.*, (2018), reported that Safeda guava fruit contained similar amount of total flavonoids with the different solvent used for extraction. Seo *et al.*, (2014) reported similar results with the guava leaves.

**Percent Free Radical Scavenging Activity (DPPH activity)-** It is generally recognized that free radicals produced in the body are partly associated with the etiology of cancers and other chronic diseases. Dietary antioxidants, capable of scavenging free radicals, are able to reduce the risk of the disease. Therefore, it is important to determine the radical scavenging effect of antioxidants in fruits. DPPH assay is generally used for the estimation of free radical scavenging activity of the antioxidants. DPPH is stable at room temperature and produces a violet solution in solvent. Antioxidant causes the discoloration of purple color to yellow color indicating the scavenging activity of the added sample. This reduction results in loss of absorbance measured at 515 nm. The DPPH values of the extracts are presented in Figure 3, for different cultivars i.e., Red guava, Lalit, Surkha, Chittidar, and Safeda, respectively. The DPPH values were highest for Safeda followed by Lalit, Chittidar, Surkha, and Red guava.

DPPH activity of guava cultivar extracts at various ripening stages in various solvents showed the similar trend in results as it was seen in TPC and TFC content. Percent free radical scavenging activity of unripe guava cultivars ranged from 16.02 to 88.46%, while for ripe guavas it was ranged between 11.23 to 70.29%. Several earlier studies

Figure 1- Effect of solvents on total phenolic content of different cultivars of guava



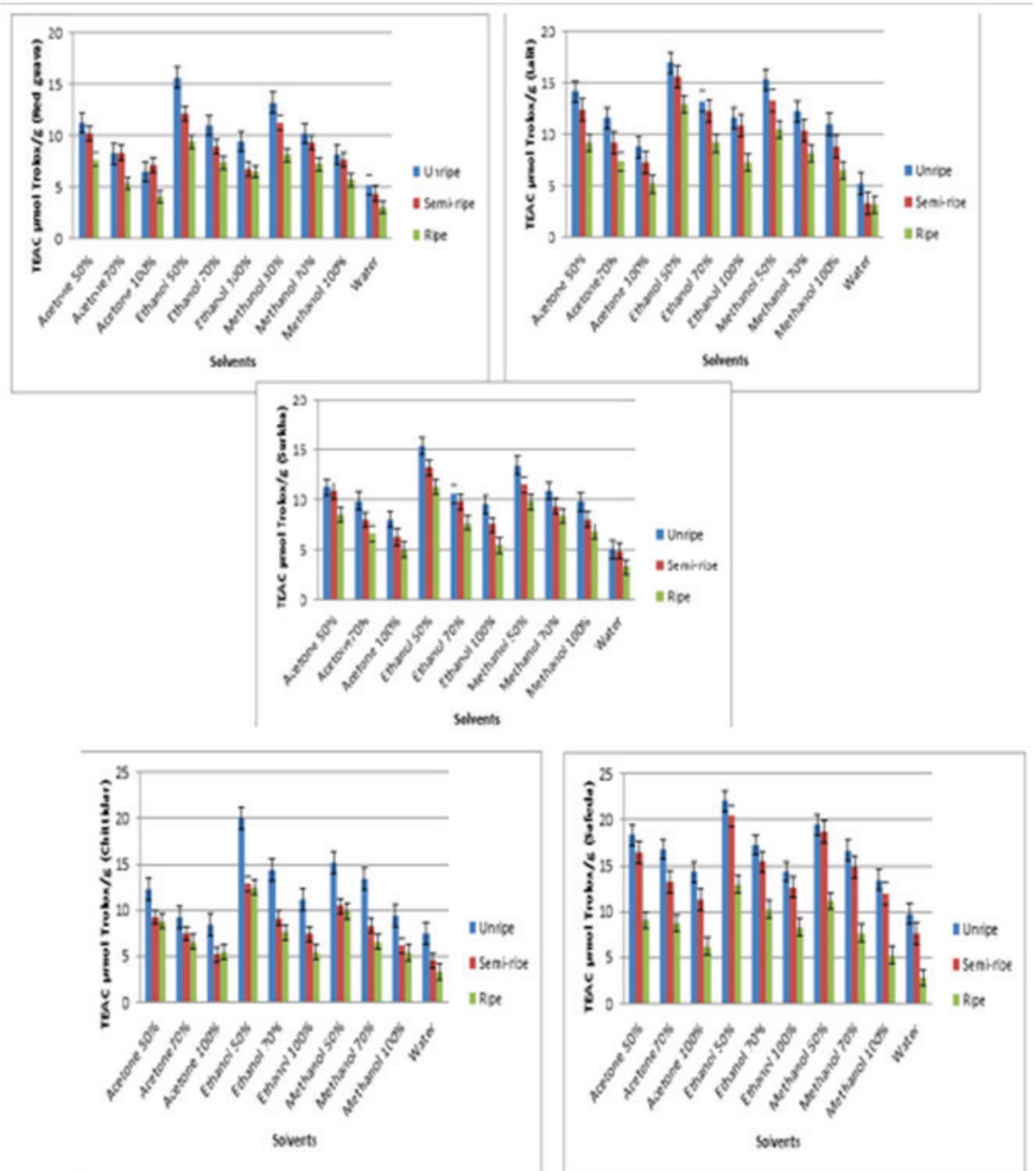
also showed that guava possess high amount of percent free radical scavenging activity (Kahkonen *et al.*, 1999, Vyas *et al.*, 2010). Flores *et al.*, (2015) examined seven edible guava (*Psidium guajava*) cultivars that varied in colour from white to pink. In the DPPH assay all four pink-pulp guavas (Barbie Pink, Homestead, Sardina 1, Sardina 2) included in the study showed higher activity than the white pulp cultivars (Yen 2 and Sayla) and less than the red pulp guava cultivar (Thai Maroon). Musa *et al.*, (2011) and Verma *et al.*, (2018) also reported that 50% ethenolic pink fleshed guava extract exhibited maximum DPPH activity.

**Antioxidant capacity determined by radical cation (ABTS<sup>+</sup>)-** ABTS<sup>+</sup> assay is based on the reaction of the ABTS<sup>+</sup> radical cation generated in the assay with the antioxidant present in the sample. This method takes comparatively less time than the other methods and it is also used to confirm the result obtained with DPPH, as both are

similar in their antioxidant mechanism. The result showed that all the cultivars and their maturity stages exhibit the antioxidant capacity but in different degrees. The values ranged between 8.11 to 55.41  $\mu\text{mol Trolox/g}$ . This activity showed inverse relation with maturity stages in all the cultivars with every season. At the ripe stage cultivars showed minimum values of ABTS<sup>+</sup>, viz., cultivar Safeda (27.50  $\mu\text{mol Trolox/g}$ ) had shown the highest cation activity as compare to other cultivars Lalit (25.6  $\mu\text{mol Trolox/g}$ ), Chittidar (24.90  $\mu\text{mol Trolox/g}$ ), Surkha (24.74  $\mu\text{mol Trolox/g}$ ), and Red guava (24.09  $\mu\text{mol Trolox/g}$ ).

Cultivar Safeda had highest ABTS<sup>+</sup> activity in all the stages followed by Lalit, Chittidar, Surkha and Red guava. The ABTS<sup>+</sup> radical scavenging abilities of guava fruit extracts are ranked as follows: 50 % ethanol > 50% methanol > 50% acetone > 70% ethanol > 70% methanol > 70% acetone > 100% ethanol > 100% methanol > 100%

Figure 2-Effect of solvent on the total flavonoid content of different cultivars of guava

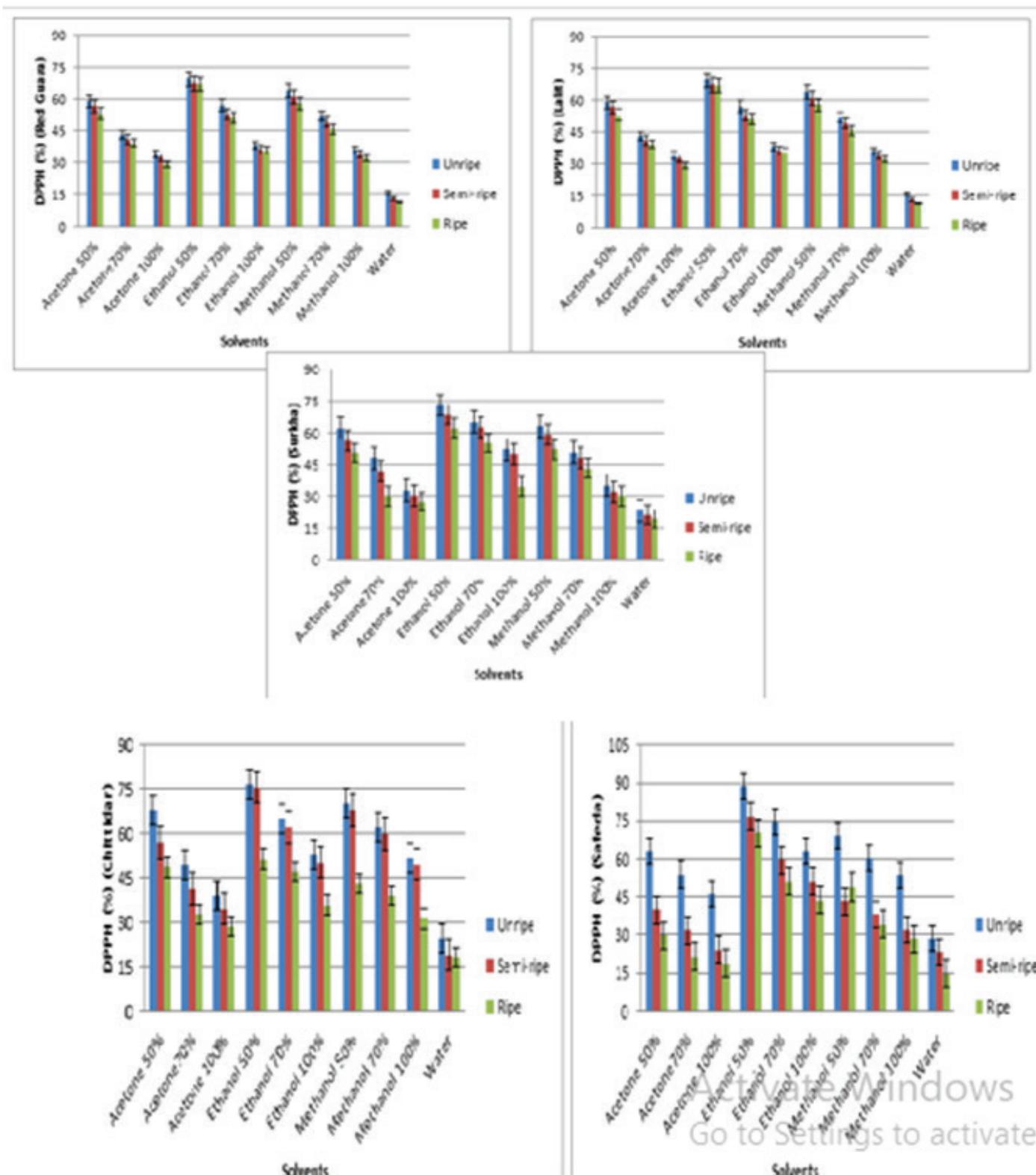


acetone > water. The results reflect that 50% ethenolic extract has maximum ABTS<sup>+</sup> activity (Figure 4). The similar decreasing trend was observed with unripe and semiripe stage of the guava fruit. A similar result was reported by Seo *et al.*, (2014); they concluded that 50% ethenolic extract of guava leaves showed maximum antioxidant activity in terms of ABTS<sup>+</sup> activity. Duda-Chodak *et al.*, (2011) had shown that the capacity to scavenge an ABTS<sup>+</sup> radical in apples

decreased during ripening.

**4.1.2.5 Ferric-reducing antioxidant power (FRAP) assay-** The FRAP values of the extracts are presented in Figure 5, for Red guava, Lalit, Surkha, Chittidar, Safeda, respectively. The highest antioxidant activity was found in cultivar Safeda followed by Lalit, Chittidar, Surkha and Red guava, respectively at all the ripening stages. 50% ethanol had shown the highest antioxidant activity as compared to other solvents showed significant difference in their FRAP

Figure 3- Effect of solvent onDPPH activity of different cultivars of guava

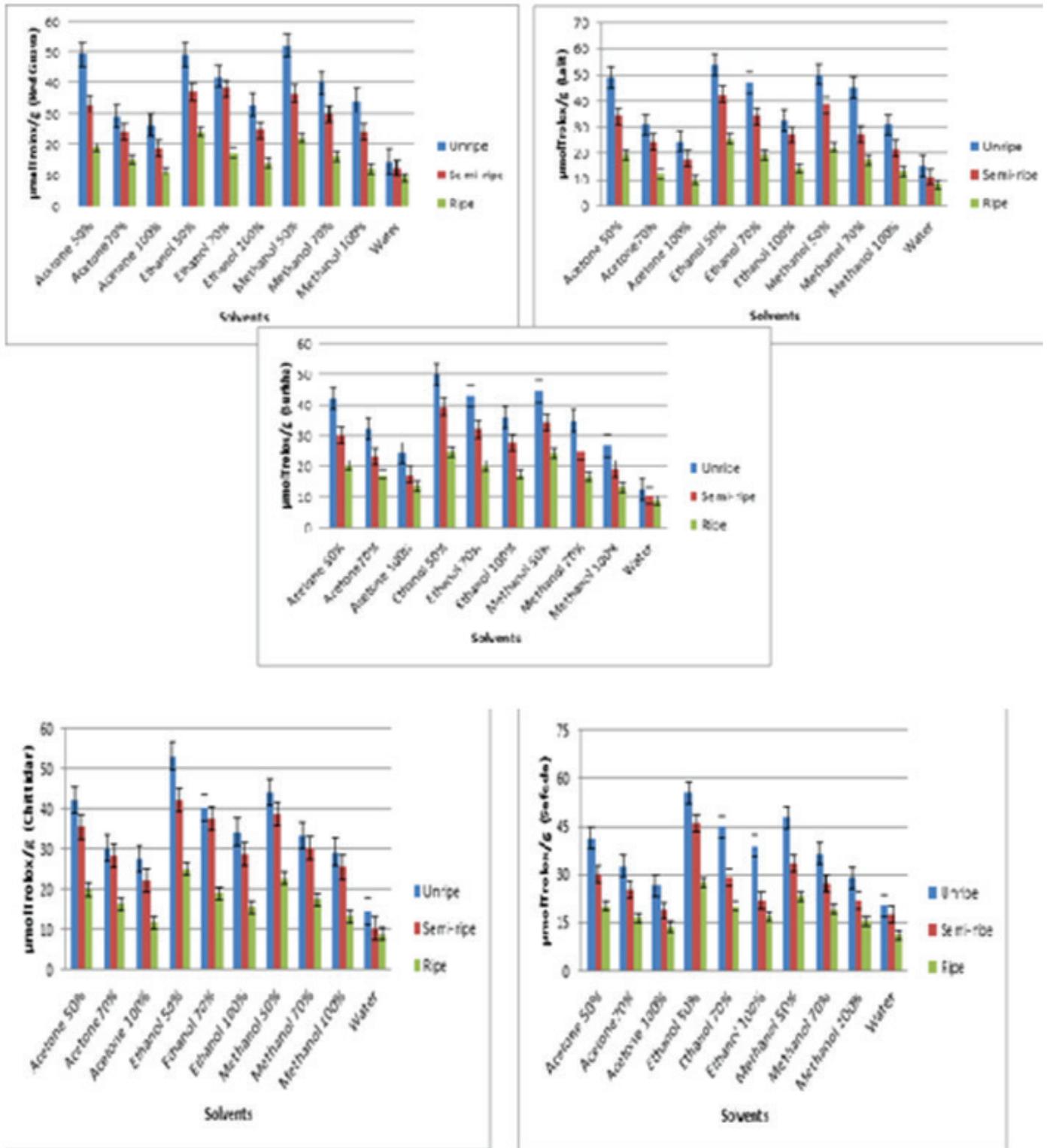


assay. The FRAP activity of guava fruit extracts are ranked as follows: 50 % ethanol > 50% methanol > 50% acetone > 70% ethanol > 70% methanol > 70% acetone > 100% ethanol > 100% methanol > 100% acetone > water. The range of FRAP activity in guava cultivars at all the ripening stages with different solvents was ranged from 18.77 to 75.36 mM Fe(II)/g for unripe fruit, from 7.89 to 40.23 mM Fe(II)/g for semi-ripe fruit, and 5.34 to 21.8M Fe(II)/g for ripe fruit.

### CONCLUSIONS

The study reveals that antioxidant activity and total phenolic content of five Guava cultivars namely Allahabad Safeda, Allahabad Surkha, Lalit, Chittidar and Apple color harvested at three fruit ripening stages (unripe, semi-ripe and ripe) and extracted by four solvents (ethanol, methanol, acetone and water) with different polarities (50%, 70% and 100%). The findings suggest that guava cultivars with maturity stages and extracting solvent affected its phenolic content and antioxidant activity significantly. Pure solvents were inefficient extraction medium for antioxidants.

Figure 4-Effect of solvent on ABTS+ activity of different cultivars of guava



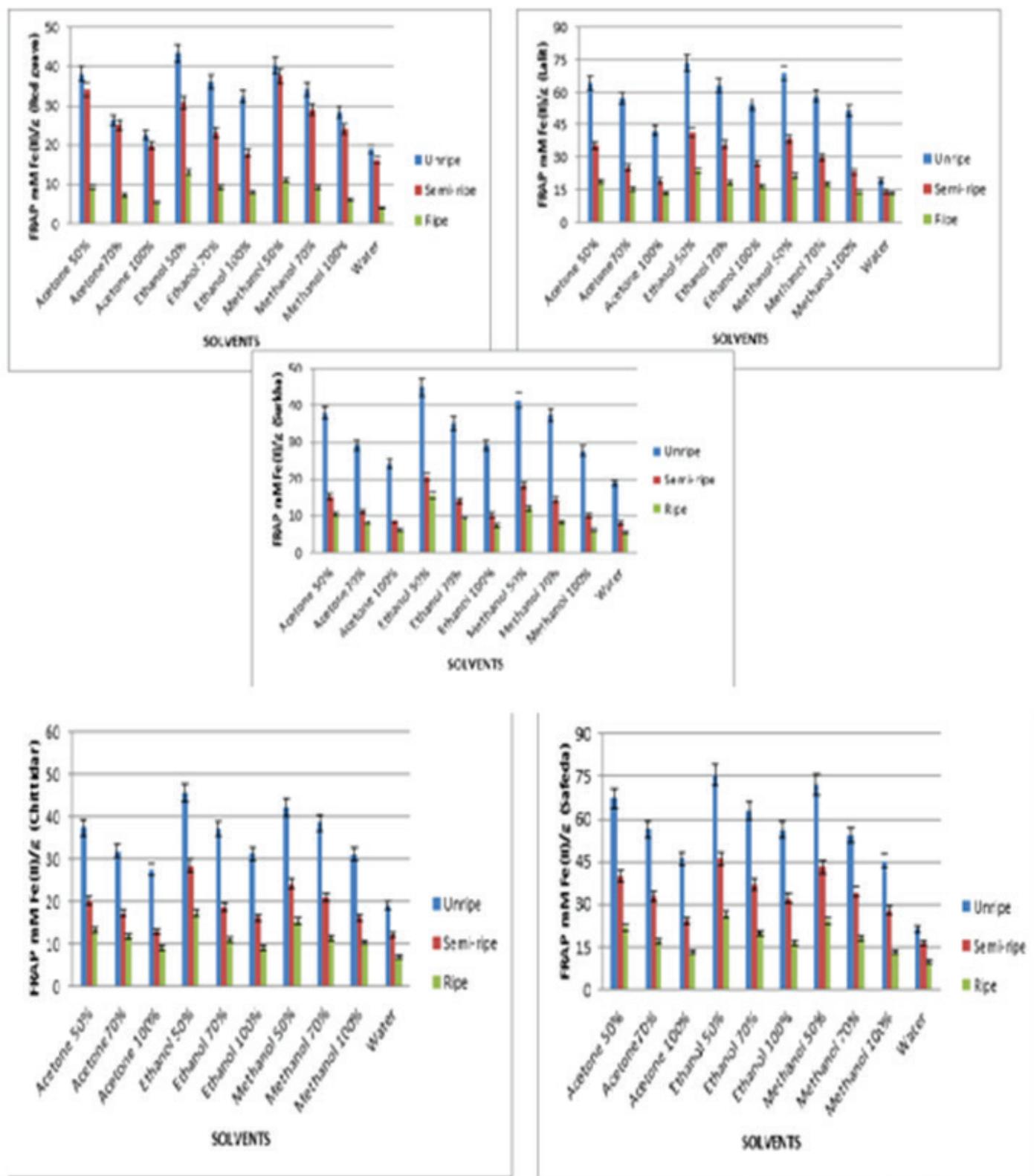
Enhanced extraction yields were obtained from solvent containing higher water concentrations and 50% ethanol is a recommended solvent for extracting antioxidants compounds from guava fruit. High correlations between phenolic compositions and antioxidant activities of guava extracts were observed. The total phenolics and antioxidant capacity decreases as the ripening proceeds (unripe>semi-ripe>ripe) in all five cultivars. This study facilitates information on the total phenolic content and antioxidant capacities of guava cultivars which is an important commercial fruit. Results obtained showed that solvent polarities and ripening stages played

the major role in extraction of polyphenolics from Guava. The significant declines in the levels of total phenolics and antioxidant capacity during fruit development suggest that the unripe fruits can also be taken for processing into nutraceutical foods.

### ACKNOWLEDGEMENT

I would like to express my deep and sincere gratitude to my research supervisor, Dr. Devinder Kaur, Assistant Professor and other authors, Center of Food Technology, University of Allahabad, for giving me

Figure 5-Effect of solvent on FRAP activity of different cultivars of guava



the opportunity to do research and providing in aluable guidance throughout this research

**REFERENCES**

Ahmad, N., F. Anwar, S.Hameed, and M.C.Boyce, (2011). Antioxidant and antimicrobial attributes of different solvent extracts from leaves and flowers of akk (Calotropis procera (Ait.) Ait. F). *Journal of Medicinal Plants Research* 5 (19): 4879-4887.

Alothman M., R.Bhat, A. A.Karim (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry* (115), 785–788.

Bahoron, T.,A.Luximon-Ramma, A. Crozier,&O. I.Aruoma, (2004). Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. *Journal of the Science of Food and Agriculture*, 84(12), 1553-1561.

- Boateng, J., M. Verghese, L. T. Walker, S. Ogutu. Effect of processing on antioxidant contents in selected dry beans (*Phaseolus* spp. L.). *LWT - Food Science and Technology* 2008, 41, 1541-1547.
- Castrejon, A.D.R., I. Eichholz, S. Rohn, L. W. Kroh, S. Huyskens-Keil, (2008). Phenolic profile and antioxidant activity of highbush blueberry (*Vaccinium corymbosum* L.) during fruit maturation and ripening. *Food Chem.*, 109, 564–572.
- Chirinos, R., H. Rogez, D. Campos, R. Pedreschi, & Y. Larondelle, (2007). Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruiz & Pavón) tubers. *Separation and Purification Technology*, 55(2), 217-225.
- Cordenunsi, B.R., J.R.O. Nascimento, M.I. Genovese, F.M. Lajolo, (2002). Influence of cultivar on quality parameters and chemical composition of strawberry fruits grown in Brazil. *J. Agric. Food Chem.* 2002, 50, 2581–2586.
- Du, Y., M. C. Wooten, & M. W. Wooten, (2009). Oxidative damage to the promoter region of SQSTM1/p62 is common to neurodegenerative disease. *Neurobiology of disease*, 35(2), 302-310.
- Duda-Chodak, A., T. Tarko, & T. Tuszyński, (2011). Antioxidant activity of apples—an impact of maturity stage and fruit part. *Acta Scientiarum Polonorum Technologia Alimentaria*, 10(4), 443-454.
- Fischer, U.A., R. Carle, D.R. Kammerer, 2011. Identification and quantification of phenolic compounds from pomegranate (*Punicagranatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD–ESI/MSn. *Food Chemistry* 127, 807–821.
- Flores, G., S. B. Wu, A. Negrin, & E. J. Kennelly, (2015). Chemical composition and antioxidant activity of seven cultivars of guava (*Psidium guajava*) fruits. *Food Chemistry*, 170, 327-335.
- Girard-Lalancette, K., A. Pichette, & J. Legault, (2009). Sensitive cell-based assay using DCFH oxidation for the determination of pro- and antioxidant properties of compounds and mixtures: Analysis of fruit and vegetable juices. *Food Chemistry*, 115, 720-726.
- Gokmen, V., A. Serpen, and V. Fogliano. (2009). Direct measurement of the total antioxidant capacity of foods: the ‘QUENCHER’ approach. *Trends in Food Science and Technology* 20 (6-7): 278-288.
- Gomez, M.L.P.A., F.M. Lajolo. (2008). Ascorbic acid metabolism in fruits: Activity of enzymes involved in synthesis and degradation during ripening in mango and guava. *J. Sci. Food Agric.* 2008, 88, 756–762.
- Guo, C., J. Yang, J. Wei, Y. Li, J. Xu, & Y. Jiang. (2003). Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutrition research*, 23(12), 1719-1726.
- Ho, R., A. Violette, D. Cressend, P. Raharivelomanana, P. A. Carrupt, & K. Hostettmann. (2012). Antioxidant potential and radical-scavenging effects of flavonoids from the leaves of *Psidium cattleianum* grown in French Polynesia. *Natural Product Research*, 26(3), 274–277.
- Joseph, B and R.M. Priya. (2011). Phytochemical and Biopharmaceutical Aspects of *Psidium guajava* (L.) Essential Oil: A Review. *Research Journal of Medicinal Plants*, 5: 432-442.
- Kähkönen, M. P., A. I. Hopia, H. J. Vuorela, J. P. Rauha, K. Pihlaja, T. S. Kujala, & M. Heinsonen (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of agricultural and food chemistry*, 47(10), 3954-3962.
- Kalt, W., C. Lawand, D. Ryan, J. E. McDonald, H. Donner. (2003). Oxygen radical absorbing capacity, anthocyanin and phenolic content of highbush blueberries (*Vaccinium corymbosum* L.), during ripening and storage. *J. Am. Soc. Hort. Sci.*, 128, 917–923.
- Lim Y.Y., T.T. Lim and J.J. Tee. (2007). Antioxidant properties of several tropical fruits: A comparative study. *Food Chemistry* (103)3, 1003–1008.
- Liu, X., M. Zhao, J. Wang, B. Yang, & Y. Jiang. (2008). Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. *Journal of food composition and Analysis*, 21(3), 219-228.
- Luximon-Ramma, A., T. Baborun, & A. Crozier. (2003). Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. *Journal of the Science of Food and Agriculture*, 83(5), 496-502.
- McCook-Russell, K. P., M.G. Nair, P. C. Facey, & C. S. Bowen-Forbes. (2012). Nutritional and nutraceutical comparison of Jamaican *Psidium cattleianum* (strawberry guava) and *Psidium guajava* (common guava) fruits. *Food Chemistry*, 134(2), 1069-1073.
- Michalak, I., K. Chojnacka & A. Saeid. (2017). Plant growth biostimulants, dietary feed supplements and cosmetics formulated with supercritical CO<sub>2</sub> algal extracts. *Molecules*, 22(1), 66.
- Misra, K., & T. R. Seshadri. (1968). Chemical components of the fruits of *Psidium guajava*. *Phytochemistry*, 7(4), 641-645.
- Musa, K. H., A. Abdullah, K. Jusoh, & V. Subramaniam. (2011). Antioxidant activity of pink-flesh guava (*Psidium guajava* L.): effect of extraction techniques and solvents. *Food Analytical Methods*, 4(1), 100-107.
- Noriham, A., S.S.W.K. Wan, S. Zainal, S.Z. Khairusy, and A. Nurain. (2012). Study on antioxidant capacity and phenolic content of various parts of wax gourd (*Benincasahispida*). *World Applied Science Journal*. 19(7), 1051–1056.
- Ojewole, J. A., E. O. Awe, & W. D. Chiwororo. (2008). Antidiarrhoeal activity of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extract in rodents. *Journal of Smooth Muscle*

- Research*, 44(6), 195-207.
- Parr, A.J., P.A.J. Bolwell. (2000). Phenols in the plant and in man: The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J. Sci. Food Agric.*, 80, 985–1012.
- Patthamakanokporn, O., P. Puwastien, A. Nitithamyong, &P.P. Sirichakwal. (2008). Changes of antioxidant activity and total phenolic compounds during storage of selected fruits. *Journal of Food Composition and Analysis*, 21(3), 241-248.
- Prasad, N. K., B. Yang, M. Zhao, B. S.Wang, F. Chen,&Y. Jiang. (2009). Effects of high-pressure treatment on the extraction yield, phenolic content and antioxidant activity of litchi (*Litchi chinensis*Sonn.) fruit pericarp. *International journal of food science & technology*, 44(5), 960-966.
- Re R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang *et al.*, (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free RadicBiol Med* 26: 1231-1237.
- Renaud, S.C., R. Gueguen, J. Schenker, A. d’Houtaud. (1998). Alcohol and mortality in middle-aged men from eastern France. *Epidemiology*, 9: 184 – 188.
- Riadh, I., C. Hdider, S. L. Marcello, I. Tlilia, and G. Dalessandro. (2011). Antioxidant activity and bioactive compound changes during fruit ripening of high-lycopene tomato cultivars. *Journal of Food Composition and Analysis* 24, 588–595.
- Seo, J., S. Lee, M. L. Elam, S. A. Johnson, J. Kang,&B. H. Arjmandi. (2014). Study to find the best extraction solvent for use with guava leaves (*Psidiumguajava* L.) for high antioxidant efficacy. *Food Science & Nutrition*, 2(2), 174-180.
- Spigno, G., L. Tramelli, &D. M. De Faveri. (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of food engineering*, 81(1), 200-208.
- Stanislaw, L. (1968). Determination of the degree of polymerization of leucoanthocyanidins. *Phytochemistry*, 7, 665–667.
- Tachakittirungrod S, F. Ikegami, and S.Okonogi. (2007). Antioxidant active principles isolated from *Psidiumguajava* grown in Thailand. *Scientia Pharmaceutica* (75), 179-193.
- Thaipong, K., U. Boonprakob, K. Crosby, L. Cisneros-Zevallos, &D. H. Byrne. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of food composition and analysis*, 19(6-7), 669-675.
- Tlilia, I., I. Riadh, C. Hdider,S. L. Marcello, and G. Dalessandro. (2011). Bioactive compounds and antioxidant activities during fruit ripening of watermelon cultivars. *Journal of Food Composition and Analysis* 24, 923–928
- Van’t Veer P., M. Janson, M. Klert, F. kok. (2000). Fruits and vegetables in the prevention of cancer and cardiovascular disease. *Public Health Nutrition*. 3, 103-107.
- Velioglu, Y. S., G. Mazza,L. Gao,&B. D. Oomah. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of agricultural and food chemistry*, 46(10), 4113-4117.
- Verma, M., G. K. Rai,&D. Kaur. (2018). Effect of extraction solvents on phenolic content and antioxidant activities of Indian gooseberry and guava. *International Food Research Journal*, 25(2).
- Viraj R., and A. Pillai. (2012). Phenolic content and antibacterial effect of guava (Allahabad safeda and Bhavnagar red) *Journal of Cell and Tissue Research* 12(2), 3255-3260.
- Vyas, N., M. Tailang,N. P. Gavatia, &B. K. Gupta. (2010). Antioxidant potential of *Psidiumguajava* Linn. *International Journal of PharmTech Research*, 2(1), 417-419.
- Wang, S.Y.; W. Zheng. (2001). Effect of plant growth temperature on antioxidant capacity in strawberry.*J. Agric. Food Chem.*, 49, 4977–4982.
- Williamson, G. and C. Manach. (2005). Bioavailability and bioefficacy of polyphenols in humans II. Review of 93 intervention studies. *Am J ClinNutr*, 81: 243S-255S.
- Yu, J., M. Ahmedna,&I. Goktepe. (2005). Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. *Food chemistry*, 90(1-2), 199-206.
- Zieliński, H., &H. Kozłowska. (2000). Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *Journal of agricultural and food chemistry*, 48(6), 2008-2016.