Contents lists available http://www.kinnaird.edu.pk/

Journal of Natural and Applied Sciences Pakistan

Journal homepage: http://jnasp.kinnaird.edu.pk/

ESTIMATION OF TOTAL PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY AND CASE STUDY OF *CARICA PAPAYA*

Faiza Hassan[1*], Ayesha Subhan[1], Umer Younas[1], Farzana Bashir[2], Fahama Zahra[3]
[1]Department of Chemistry, The University of Lahore, Lahore 54000, Pakistan

Article Info

*Corresponding Author
Email: faiza.hassan@chem.uol.edu.pk

Abstract

The extraction of bioactive compounds from botanical sources are beneficial for human health. *Carica Papaya* have become well known for antioxidant components, can be used for the treatment of several diseases such as cancer, wound healing, glandular tumors, eczema, cutaneous, corns, warts, sinuses, tubercles, general debility, dyspepsia, blood pressure, constipation, expel worms and stimulate reproductive organs and many other diseases, as a result *Carica papaya* can be regarded as a Nutraceutical. The purpose is to determine the total phenolic content and antioxidant activities in extracts of *Carcia papaya fruit* and also role play in wound healing. Competency of the solvents (water, methanol, acetone, chloroform, ethanol) were evaluated by analyzing extracts of ripe and unripe *C. papaya* for their total phenolic and radical (DPPH) scavenging potential and FTC methods for measured to inhibit lipids oxidation. The testified methods were found having similar extraction potential, whereas significant difference was recorded among solvents regarding their extraction efficiency. Different solvents extracts were also compared for the ripening and unripening stages of papaya the maximum value of antioxidant was calculated in ripe. *C. papaya* also showed positive response in wound healing. Fruits are the principle source of phenolics, which is based on bioactive compounds. In vitro and in vivo testified that the importance of phenolics compounds in preventing the human fatal or degenerative diseases. A wide range of antioxidant potential of *Carica papaya* fruit. The all parts of papaya plant has its own pharmaceutical value. In the *Carica papaya* the presence of vitamins, enzymes, makes it a nutraceutical plant. In research and food industry the antioxidant properties of methanolic extract of *Carica papaya* has recently been develop great interest, for the reason that of its possible use as natural supplements which occurred. In this study showed that the high phenolic content in ripe papaya it is also be used in pharmaceutical and food industries. *Carica papaya* is the good source of antioxidant potential and used in wound healing properties.

Keywords
Papaya, antioxidant, wound healing, phenolic content, solvent effect.

1. Introduction:

Free radicals, reactive oxygen and nitrogen species are continuously produced in human body during normal cellular metabolism, liver functions and mitochondrial respiratory system (Iqbal *et al.,* 2012). Role of free radicals and reactive species in causing oxidative stress in human body after damaging biomolecules (lipids, DNA, proteins etc.) is well known. Oxidative stress has been reported as fundamental mechanism for the development of multiple health disorders (neurodegenerative, autoimmune), infections and diseases (Alzheimer's disease, Parkinson's disease and Ulcers) (Repetto & Llesuy., 2002; Aruoma, 2003; Jacobo & Cisneros., 2009). In addition, reactive species may also initiate multi-stage carcinogenic process, starting with the DNA damage, which progressively leads to dysplastic cellular appearance, deregulated cell growth and finally carcinoma (Tsao *et al.*, 2004).

In human body, naturally occurring defense mechanism equipped with enzymatic and non-enzymatic antioxidants fights against reactive species to reduce their effects. However, in state of oxidative stress, built in antioxidant system sometime requires external aid of antioxidants to compete with excessive amount of free radicals and reactive species. Initially synthetic compounds were used as antioxidant supplements, but reports discussing their carcinogenic nature motivated researches to search antioxidants from natural sources. As a result, many botanical materials (fruits, vegetables, medicinal plants, herbs, shrubs) have been reported as potent sources of antioxidants such as polyphenols, vitamins and tocopherols (Landrault *et al.,* 2001; Shahidi, 2000; Wilson, 1999). This may also be helpful in prevention and treatment of cancer (Lee *et al.,* 2004).

Phytochemicals possessing with maximum antioxidant properties needs to be recovered carefully from botanical matrix prior qualitative, quantitative and bioactivity analysis. For comprehensive extraction of target compounds, selection of proper extraction method, solvent and conditions are of much importance. Many reports have been published discussing effect of solvent polarity on recovery of antioxidant and anticancer bioactive from botanical materials (Meneses *et al.*, 2013). Frequently used solvents range from polar solvents (ethanol, methanol, acetone, ethyl acetate) to non-polar solvent system (Dichloromethane and n-hexane). Solvents differ in their extraction ability, as low polarity solvents like acetone may extract alkaloids, glycosides and aglycones whereas high polarity solvents such as methanol may solubilize amino acids, sugars and glycosides (Houghton & Raman., 1998). Furthermore, availability of these compounds to solvent system strongly depends upon extraction techniques. Extracts prepared through different techniques or media may vary widely for their contents of bioactive phytochemicals that may exhibit specific biological activity at their best. Therefore, these extracts are individually analyzed using different assays and established instrumental methods (Hussain *et al.,* 2012; Musa *et al.,* 2011).

Carica papaya belongs to the genus Carica, family *Caricaceae* and the subclass dicotyledoneae, commonly known by the names of pawpaw or papaw (Delbridge & Delbridge., 1981). It was first cultivated in Mexico several centuries ago and now it is grown round the world in tropical regions such as Africa, India and Srilanka. In Pakistan, province of Punjab and Sindh especially Malir area of Karachi and coastal areas of Sindh province possess lush green orchards of papaya (Oad *et al.,* 2011). Papaya is a good source of Vitamin A, C, E and K, as well as folate and fibre (Milind, 2011). Papaya fruit have shown many biological activities due to the presence of active components including papine, chymopepine, ascorbic acid, flavonoids and cynogenicglucosidase etc. In recent studies *Carica papaya* extracts has been reported as strong anti-dengue tonic that is helpful in recovering the white blood cell quickly after infection (Ahmad *et al.,* 2011).

Recently, a report has been published discussing anticancer activity and effect of extraction conditions on antioxidant potential of *C. papaya* (Vuong *et al.,* 2013). To the best of our knowledge, no report have been published so far, the study aim to discussing effect of solvent systems on antioxidant potential of *C. papaya* ripe and unripen stage. The maximum amount of phenolic compounds and antioxidant activity of papaya fruit extracts, as well as their physicochemical properties at ripening stages. Therefore, present study was designed to determine the effect of solvents (water, acetone, ethanol, methanol, chloroform) on extraction of antioxidant compounds (phenolics) and radical (DPPH, FTC) scavenging potential of *C. papaya*. These investigations will be helpful in securing maximum benefits associated with bioactive compounds present in papaya. It will also play a key role in development of anticancer medicine, functional food and nutraceuticals and also play very important role in wound healing (Jung *et al.,* 2006).

2. Material And Methods

2.1.Collection of Carica papaya fruit

Carica papaya (Variety Golden) was collected from Local market of Lahore, Punjab.

2.2.Preparation of Carica papaya extract

Before analysis, every sample were instantly washed several times with tap water. The samples were then individually prepared where the edible portion (ripe and unripe) were peel out and small pieces then grinded. After this took 10g papaya (ripe, unripe) from paste dissolved in (methanol, ethanol, acetone, chloroform, water). After this shake it and put it into dark botel at 200rpm for 2 hours and continuous stirring in a dark bottle using an orbital shaker .Then after 2 hrs. Centrifuge at 1000rpm for 15min. filtered the mixture through a filter paper (Whatman No. 4).

2.3. Quantification assays

2.3.1. Determination of total phenolic content

Total phenolic contents were determined following Folin- Ciocalteu reagent assay and was reported (Iqbal *et al.,* 2012). The reaction mixture was prepared by mixing 2 mg of papaya extract and 100 µL of freshly prepared 0.5 N Folin- Ciocalteu reagent. The prepared mixtures were allowed to stand in dark for 15 min followed by addition of 2.5 mL sodium carbonate (6%) and resultant mixture was incubated in dark for 30min. The absorbance was recorded at 725 nm using UV visible spectrophotometer (CECIL, Milton Technical Centre, Cambridge UK). Ascorbic acid was used as standard and results were calculated as of ascorbic acid equivalents mg $g^{-1}$of extract (Annegowda *et al.,* 2012).

2.3.2. Determination of radical scavenging potential

2.3.2.1.DPPH radical scavenging assay

The capacity of the extracts to scavenge 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical was determined according to the method reported in literature (Ding *et al.,* 2002). Diluted papaya extract (0.2 mL) was added to 3.9 mL of methanolic solution of DPPH radical (25 mg/mL). The mixture was shaken vigorously and left in the dark for 30min. The absorbance of the mixture was recorded at 500 nm against a blank of absolute methanol without $DPPH^•$. The results were calculated as percentage remaining of $DPPH^•$, which were used to compare the radical scavenging potential of *C. papaya* fruit extracts.

2.3.3. Estimation for lipid oxidation

2.3.3.1.FTC (Ferric thiocyanate method)

In this method, hydro peroxide produced by linoleic acid added to the reaction mixture, which has been oxidized by air during the experimental period is indirectly measured (Wijekoon *et al.,* 2011). 2ml of sample (methanol extract)+2.5%linolenic acid in 99.8% ethanol took 2.05ml+4ml of 0.05 mol after this added 1.95ml water in flask and placed in a rotary incubator (150r/min,40 degree centigrade)in a dark place. To measure antioxidant value 0.1ml of above sample mixture in test tube then added 9.7ml of 75% ethanol +0.1ml of 30% ammonium thiocyanate and 0.1ml of 2×10^{2} mol /L ferric chloride in 3.5% HCL Three min after the addition of ferrous chloride to the reaction mixture, the absorbance was measured at 500nm. Measurement were taken every 24hr until the absorbance of the control reached its maximum value this mixture also prepared without linoleic acid and a negative control (Lee *et al.,* 2004).

Sample preparation: Took 0.5ml of sample (ripe, unripe, vitamin E) then added 0.5ml linoleic acid,0.5ml water and add 1ml phosphate buffer after this covered at 40 degree centigrade put into oven. Took 0.1ml from the above soln. then added 9.7ml of 75% ethanol, 0.1ml ammonium thiocyanate, and 0.1ml of $Fecl_2$ in HCl soln.

Control: 1ml buffer, 0.5ml water put at 40 degree centrigrade in oven after this took 0.1ml of above soln then added 9.7ml of 75% ethanol 0.1ml ammonium thiocyanate, 0.1ml of $Fecl_2$ in HCl soln.

Blank: Add 0.5ml of methanol, 0.5ml of water, 1ml buffer and then similar process of the above (Zhou *et al*., 2004)

3. Results and Discussion

3.1.Total phenolic content

Many reports have revealed that antioxidant activity of botanical materials mainly depends upon their phenolic constituents (Kong *et al*., 2012). Phenolic compounds are secondary metabolites, which are well known for their free radical scavenging potential due to their hydrogen donating and metal chelating abilities (Gai *et al.*, 2013; Mathew & Abraham., 2006).

3.2.Estimation of total phenolic content (TPC)

In current study, TPC was estimated by the FC reagent method using ascorbic acid as standard. Formation of blue color indicated the presence of phenolics and absorption was recorded at 725nm.

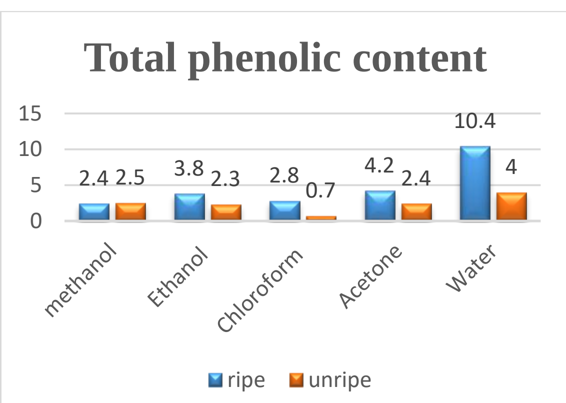


Figure 1: Total phenolic content in (µg/g) of ripe and unripe *C. papaya* fruit as influenced by the solvents

In this result observed that the high polarity solvents use recovery of TPC was also improved and the highest concentration of phenolics was found in water extracts of ripe papaya and unripe papaya; approving the ability of water to solubilize a larger fraction of the phenolic components present in papaya (Jayaprakasha *et al.,* 2008). Water showed comparable potential as compare to the other solvents. All these observations recommend that most of the phenolic compounds are highly polar in polar solvents. The range of polarity of solvents showed in ripe papaya is water>acetone>ethanol>chloroform>methanol.

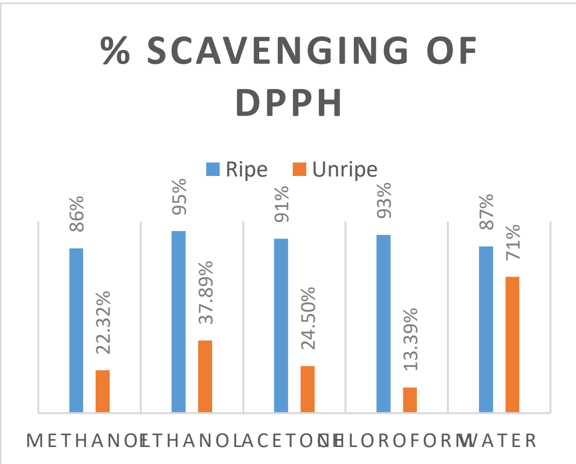
In some other reports the content of phenolic compounds in different solvents (methanol, acetone, and ethanol) the high content showed in methanol extract as compare to other solvents and the phenolic content in ripe were more than the unripe (Sultana *et al.*, 2009).

3.3.Evaluation of antioxidant activity

3.3.1. DPPH radicals scavenging activity:

DPPH radical scavenging antioxidant assay have been used in many studies for the evaluation of antioxidant potential. At room temperature DPPH is stable free radical and takes a hydrogen / electron radical to become a stable molecule (Hemwimon *et al.*, 2007). The DPPH radical reduction ability is evaluate by reduce in its absorbance at 500 nm, prompted by antioxidants. Antioxidants is caused the decrease in absorbance of DPPH radical. Because of the reaction progresses between antioxidant radicals and molecules, which results in the scavenging of the radical by donation of hydrogen. In this reaction that the change in colour from purple to yellow (Mosmann, 1983). Therefore, DPPH is normally used to evaluate the antioxidantive activity. The results showed that the extract of ripe and unripe papaya with various solvents can be seen in Figure 2.

Figure 2: DPPH radical scavenging potential of extracts prepared using different extraction methods and solvents

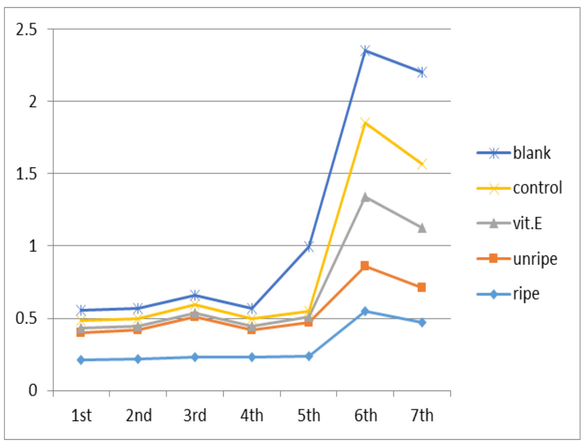


In this Figure 2, the % scavenger of the best extraction shown high polarity solvents being such as ethanol, chloroform, acetone, methanol and water have been used for the determination of antioxidant potential. The % scavenging effect is higher in ripe as compare to the unripe papaya.

3.3.2. FTC (Ferric thiocyanate Method)

FTC method evaluate that the amount of peroxide produced at the initial stage of lipid oxidation, The FTC assay was carried out as describe by Kikuzaki (Prasad *et al.*, 2009). In the FTC method, all extracts showed strong antioxidant potential with percent inhibition consecutive seven days reading. By measuring the absorbance of hydroperoxides of linoleic acid this method was used to evaluate the level of lipid peroxidation .In this method, the concentration of peroxide decreases as the antioxidant activity increases (Leng *et al.*, 2005). The antioxidant activity of the dimethyl sulfite (DMS), ethyl acetate (EA), hexane and methanol extracts of red papaya peel and flesh were measured using ferric thiocyanate method (FTC) (Jiang *et al.*, 2011). Samples were oxidized when stored for seven days at 40-45°C. Initially the methanol fruit extracts had showed the highest antioxidantive activity. The comparison of sample extracts between α- tocopherols, BHT and BHA showed has a significant difference ($p<0.05$) in total antioxidant activity compared to the control. After seven days, it had been shown that all samples effectively inhibit linoleic acid oxidation (Alam *et al.*, 2013).

Figure 3: Antioxidant activity of five methanolic plant extracts as measured by the FTC method at 500 nm and compared to standards (Vitamin E).



This study shows that the antioxidant potential is high in unripe papaya fruit as compare to the ripe papaya fruit. This method has not been used previously for papaya pulp. The increased absorbance value of step by step and reached maximum levels on day 6 and finally dropped on seven days. The blank had the highest ($p < 0.05$) absorbance value (1.12) followed by papaya unripe extract is high antioxidant as compare to the ripe papaya. The antioxidant activity of unripe is (61.23%) and ripe has (25.94%).

3.4.Wound healing:

For ago, in healthcare systems plants playing a major role and have been used to control various diseases. The use of plant extracts or plant derived compounds in the treatment and wound healing have been well recognized. In the same way, in various plant extracts the presence of effective antioxidants is well recognized. Many plants or plant derivative compounds having high levels of antioxidant potential, also show wound curing properties. Papaya is very effective in helping wound healing was studied extensively both *in vitro* and *in vivo.* Papaya extract had been shown to promote wound-healing in incision type and open wounds as represented by a greater collagen content and thickness of epithelium (outer layer of skin).Antioxidants are being first pathway to protect against oxidative stress damage, and very beneficial for health and safety. Because many aspects can take part in oxidative stress, individual estimation of liability becomes important (Süntar *et al.,* 2012).

3.5.Case Study of Carica Papaya

The case study is on 20 patients of the cesarean section gaped wound healing. In this study wounds washed with normal saline and then filled with grinded papaya. Wounds covered with dressing for 48 hours. This process was repeated till the wound clean after this healthy granulation tissue seen. Total duration of hospital stay and the efficacy parameters that needed to develop healthy granulation tissue studied (Sultana, 2012). The effectiveness and well-being of papaya dressing with particular wound dressing in surgical fetus delivery gaped wounds.

3.5.1. Study Design

Randomized, qausi experimental study.

3.5.2. Background

Gynecology Unit 1, Sir Ganga Ram Hospital Lahore Pakistan, within the duration of six months.

4. Conclusion

Papaya dressing is safe and effective for gaped wound healing and it acts as antioxidant. The benefit is that there is no bleeding, anesthesia requirement and no pain. Moreover it is widely available cost effective.

5. References

Ahmad, N., Fazal, H., Ayaz, M., Abbasi, B. H., Mohammad, I., & Fazal, L.(2011). Dengue fever treatment with *Carica papaya* leaves extracts. *Asian Pacific journal of tropical biomedicine,* 1, 330-333.

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21, 143-52.

Annegowda, H., Mordi, M., Ramanathan, S., Hamdan, M., & Mansor, S. (2012). Effect of extraction techniques on phenolic content, antioxidant and antimicrobial activity of Bauhinia purpurea: HPTLC determination of antioxidants. *Food Analytical Methods* , 5, 226-233.

Aruoma, O.I. (2003). Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 523, 9-20.

Delbridge, A.& Delbridge, A.(1981). *The macquarie dictionary*. Cambridge Univ Press, 11-14.

Ding, W. J., Hasegawa, T., Peng, D., Hosaka, H., & Seko, Y. (2002). Preliminary investigation on the cytotoxicity of tellurite to cultured HeLa cells. *Journal of trace elements in medicine and biology*, 16, 99-102.

Gai, Q. Y., Jiao, J., Mu, P.S., Wang, W., Luo, M., Li, C. Y., Zu, Y. G., Wei, F. Y., & Fu, Y. J. (2013). Microwave-assisted aqueous enzymatic extraction of oil from *Isatis indigotica* seeds and its evaluation of physicochemical properties, fatty acid compositions and antioxidant activities. *Industrial Crops and Products*, 45, 303-311.

Hemwimon, S., Pavasant, P., & Shotipruk, A. (2007). Microwave-assisted extraction of antioxidative anthraquinones from roots of *Morinda citrifolia*. *Separation and purification technology* , 54, 44-50.

Houghton, P. J., & Raman, A.(1998). Crude fractionation procedures. *Laboratory Handbook for the Fractionation of Natural Extracts*. Springer, 54-65.

Hussain, A., Chatha, S. S., Noor, S., Khan, Z., Arshad, M., Rathore, H., & Sattar, M. A.(2012). Effect of Extraction Techniques and Solvent Systems on the Extraction of Antioxidant Components from Peanut (Arachis hypogaea L.) Hulls. *Food Analytical Methods*, 5, 890-896.

Iqbal, S., Younas, U., Chan, K. W., Sarfraz, R. A., & Uddin, M. K.(2012). Proximate composition and antioxidant potential of leaves from three varieties of Mulberry (*Morus* sp.): a comparative study. *International journal of molecular sciences*, 13, 6651-6664.

Iqbal, S., Younas, U., Chan, K. W., Zia, U. H., & Ismail, M.(2012). Chemical composition of Artemisia annua L. leaves and antioxidant potential of extracts as a function of extraction solvents. *Molecules*, 17, 6020-6032.

Jacobo, V. D.,& Cisneros, Z. L.(2009). Correlations of antioxidant activity against phenolic content revisited: a new approach in data analysis for food and medicinal plants. *Journal of food science,* 74, 107-113.

Jayaprakasha, G. K., Girennavar, B., & Patil, B. S. (2008). Antioxidant capacity of pummelo and navel oranges: Extraction efficiency of solvents in sequence. *LWT-Food Science and Technology,* 41, 376-384.

Jiang, C., Wang, M., Liu, J., Gan, D., & Zeng, X.(2011). Extraction, preliminary characterization, antioxidant and anticancer activities *in vitro* of polysaccharides from *Cyclina sinensis*. *Carbohydrate polymers*, 84, 851-857.

Jung, C. H., Seog, H. M., Choi, I. W., Park, M. W., & Cho, H. Y.(2006). Antioxidant properties of various solvent extracts from wild ginseng leaves. *LWT-Food Science and Technology,* 39, 266-274.

Kong, K.W., Mat, J. S., Aminudin, N., Ismail, A., & Abdul, A. A.(2012). Antioxidant activities and polyphenolics from the shoots of *Barringtonia racemosa* (L.) Spreng in a polar to apolar medium system. *Food Chemistry*, 134, 324-332.

Landrault, N., Poucheret, P., Ravel, P., Gasc, F., Cros, G., & Teissedre, P. L.(2001) Antioxidant capacities and phenolics levels of French wines from different varieties and vintages. *Journal of Agricultural and Food Chemistry,* 49, 3341-3348.

Lee, J. Y., Hwang, W. I., & Lim, S, T. (2004). Antioxidant and anticancer activities of organic extracts from *Platycodon grandiflorum* A. De Candolle roots. *Journal of ethnopharmacology,* 93, 409-415.

Lee, J., Koo, N., & Min, D.(2004). Reactive oxygen species, aging, and antioxidative nutraceuticals. *Comprehensive reviews in food science and food safety,* 3, 21-33.

Leng, B., Liu, X. D., & Chen, Q. X.(2005). Inhibitory effects of anticancer peptide from Mercenariaon the BGC-823 cells and several enzymes. *FEBS letters* , 579, 1187-1190.

Mathew, S., & Abraham, T. E.(2006). In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food and Chemical Toxicology* , 44, 198-206.

Meneses, N. G., Martins, S., Teixeira, J. A., & Mussatto, S. I.(2013). Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. *Separation and Purification Technology* , 108, 152-158.

Milind, P. (2011). Basketful benefits of papaya. *International Research Journal of Pharmacy*, 2, 6-12.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods* ,65, 55-63.

Musa, K., Abdullah, A., Jusoh, K.,& Subramaniam, V.(2011). Antioxidant Activity of Pink-Flesh Guava (Psidium guajava L.): Effect of Extraction Techniques and Solvents. *Food Analytical Methods , 4*, 100-107.

Oad, F., Lakho, A., Khan, A., Ansari, A. H., Sheikh, F., & Khail, M.(2001). Economics of Papaya in Malir, District Karachi, Pakistan. *International Journal of Agriculture and Biology* 3, 477-481.

Prasad, K. N., Hao, J., Shi, J., Liu, T., Li, J., Wei, X., Qiu, S., Xue, S., & Jiang, Y. (2009). Antioxidant and anticancer activities of high pressure-assisted extract of longan (*Dimocarpus longan* Lour.) fruit pericarp. *Innovative Food Science & Emerging Technologies*, 10, 413-419.

Repetto, M., & Llesuy, S. (2002) Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Brazilian journal of medical and biological research ,* 35, 523-534.

Shahidi, F. (2000). Antioxidant factors in plant foods and selected oilseeds. *BioFactors*, 13, 179-185.

Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, 14, 2167-2180.

Sultana, R. (2012). Enzymatic Wound Debridement; Role of Papaya in the Management of Post Cesarean Gaped Wounds. *Annals of King Edward Medical University,* 18, 355-361.

Süntar, I., Akkol, E. K., Nahar, L., & Sarker, S. D. (2012). Wound healing and antioxidant properties: do they coexist in plants? *Free radicals and antioxidants*, 2, 1-7.

Tsao, A. S., Kim, E. S., & Hong, W. K.(2004). Chemoprevention of cancer. *A Cancer Journal for Clinicians*,, 54, 150-180.

Vuong, Q. V., Hirun, S., Roach, P. D., Bowyer, M. C., Phillips, P. A., & Scarlett, C. J. (2013). Effect of extraction conditions on total phenolic compounds and antioxidant activities of Carica papaya leaf aqueous extracts. *Journal of Herbal Medicine,* 3, 104-111.

Wijekoon, M., Bhat, R., & Karim, A. A.(2011). Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (*Etlingera elatior* J.) inflorescence. *Journal of Food Composition and Analysis*, 24, 615-619.

Wilson, T. (1999). Whole foods, antioxidants and health. *Antioxidants in Human Health and Disease ;* CAB

International, Wallingford, UK, 141-150.

Zhou, K.,Laux, J.J., & Yu, L.(2004). Comparison of Swiss red wheat grain and fractions for their antioxidant properties. *Journal of Agricultural and Food Chemistry*, 52, 1118-1123.