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Evaluation of antioxidant potential of mango after formalin treatment during preservation

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Abstract

Free radicals are producing continuously inside the living cell as a part of normal metabolic process and responsible for the generation of various types of disease such as cancer, cardiovascular disease, neurological disease, pulmonary disease, rheumatoid arthritis, nephropathy, ocular disease etc. Antioxidants are continuously counterbalancing the oxidative radical by breakdown or neutralizing the free radical. Various fruits and plant parts possesses antioxidant activity for example our studied sample mango has antioxidant property but when it treated with formalin it markedly reduces antioxidant potential of it. In phosphomolybdate assay it is found that normal mango peel contains more antioxidant activity than mango flesh, but treatment with formalin, antioxidant potential decreases in both peels and fleshes. Similar result was found in iron reducing power assay and DPPH radical scavenging assay where it is found that normal mango peel has almost similar scavenging property as compared to standard BHT. Normal mango peel (NP) exhibits very high radical scavenging activity (IC_{50} is 4.2). At 100 µg/ml radical scavenging activity of normal mango peel (NP) is 93.79%, but formalin treated mango peel (FP) at same concentration possess scavenging property 76.36%, where as standard antioxidant scavenges about 93.95%. Scavenging power of normal mango flesh (NF) is 39.57% and in formalin treated mango flesh (FF) scavenging potential is only 7.12% at 100 µg/ml.
Keywords: *M. indica*, Antioxidant assay, Reducing power, DPPH

1.Introduction

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease [1]. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems form, a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [2].

Mango, (*Mangifera indica*) is a juicy stone fruit belonging to the genus Mangifera and cultivated mostly for edible fruits, native to South and Southeast Asia. The Food and Agriculture Organization of the United Nations estimates worldwide production at nearly 38,600,000 tonnes in 2011 [3]. From newspaper report it is observed that mango along with other fruits such as litchi, blackberry are regularly treated with formalin (37% solution of

formaldehyde) to keep them stay for long time. A survey in 26 markets in Dhaka city, Bangladesh by environment protective agency "Save the Environment Movement" (SEM) during June 1 to 10, 2013 found that around 94% mangoes and 100% blackberries and litchis are formalin-tainted [4]. A similar result is found in the following year, tests conducted by Poribesh Bachao Andolon (POBA) from June 1 and June 10, 2014 show that, all the black berries and 95% litchis sold in 35 city kitchen markets of Dhaka city contained formalin [5]. Scientific scholars suggest that consumption of formalin directly through food can cause different types of cancers [6-7] especially the lung cancer [8].

Recently, International Agency for Research on Cancer [9] has classified formaldehyde as a Group 1 carcinogenic to humans. Exposed with formalin a person experienced various side effects such as vomiting, abdominal pain, nodal tachycardia, acute mucus membrane irritation, dermatitis, pain and burning sensation, tearing eyes, sneezing, coughing, decreased olfactory functioning, allergic asthma [10]. There are also reports of irritated skin, heartburn, tremor, body sores, chest pain, lethargy, and loss of appetite [11-14]. Many reports indicate that chronic exposure to formaldehyde increases the chances of headache and dizziness by 30–60% [15-18]. The majority of the studies show that long term exposure can decrease the number of WBC and possibly lower platelet and haemoglobin counts [19-21]. Workers exposed to formaldehyde showed an increase in DNA damage in peripheral lymphocytes measured by single cell gel electrophoresis [22-25].

There are lots of antioxidant works have been carried out on *M. indica*, [26-30] but there is no data available about change of antioxidant activity after formalin treatment of it. The objective of this study is to find out the effects of formalin on antioxidant properties of mango peel and flesh.

2. Material and Methods

2.1 Collection of plant material

About 10 kg raw mango was collected from the garden, washed out in distilled water and shed dried. The mango was divided into two groups, one group is treated with formalin for 7 days and other group was kept as normal. The formalin solution was sprayed by the spray gun. Both groups of mangoes were peeled off and peels and fleshes are oven dried at 55°C, then ground into coarse powder. The samples are then extracted by ethanol under sonication bath and filtered. The filtrate was then concentrated with a rotary evaporator under reduced pressure at 50°C.

2.2 Phosphomolybdate assay (total antioxidant capacity)

The assay was based on the reduction of Mo (VI)- Mo(V) by the extracts and subsequent formation of a green phosphate/Mo(V) complex at acidic pH [31]. Each sample (0.5 ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate). The tubes were incubated at 95°C for 90 min. The mixture was cooled to room temperature and the absorbance of the solution was measured at 695 ηm against a blank. The antioxidant activity was expressed as the absorbance of the sample.

2.3 Ferric reducing power assay

The reducing power of the normal and formalin treated mango was determined according to method as previously described by Oyaizu M [32]. Aliquot (0.25 ml) of samples solution at different concentrations (6.25 to 100 µg/ml) was mixed with 0.625 ml of 0.2 M phosphate buffer (pH 6.6) and 0.625 ml of 1% (w/v) solution of potassium ferricyanide. After mixing well, all the mixtures were incubated in a water bath at 50°C for 20 min. Then, 0.625 ml of 10% (w/v) trichloro- acetic acid solution was added and the mixture was then centrifuged at 3000 rpm for 10 min. A 1.8 ml of the supernatant was combined with 1.8 ml of distilled water and 0.36 ml of a 0.1% (w/v) solution of ferric chloride. The absorbance was measured at 700 ηm with a spectrophotometer. Ascorbic acid was used as positive control.

2.4 DPPH radical scavenging activity

Radical scavenging activities of normal and formalin treated mango extracts were determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay [33-34] with some modifications. Briefly, sample solution with different concentrations (6.25 to 100 µg/ml) was mixed with 0.3% of DPPH methanol solution. The reaction mixtures were incubated at room temperature and allowed to react for 30 minutes in the dark. After 30 min, the absorbance values were measured at 517 ηm and converted into percentage of antioxidant activity. Butylated hydroxy toluene (BHT) was used as a positive standard control. The percentage of inhibition of DPPH (%) was calculated as follows:

$$\% \text{ inhibition of DPPH} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

The concentration of sample required to scavenge 50% of the DPPH free radical (IC_{50}) was determined from the curve of % inhibitions plotted against the respective concentration.

3. Results

3.1 Phosphomolybdate assay (total antioxidant capacity)

The results of total antioxidant activity of extracts of peels and fleshes of both normal and formalin treated are presented in the figure 1. The result demonstrated that normal mango peel exhibit the maximum antioxidant activity in a concentration dependent manner. Formalin treated extract also exhibit the activity to moderate extent. The flesh of both normal and formalin treated extract exhibit very mild antioxidant activity. The activity of different extract and reference are exhibit in the following order:

CAT> NP> FP> NF> FF

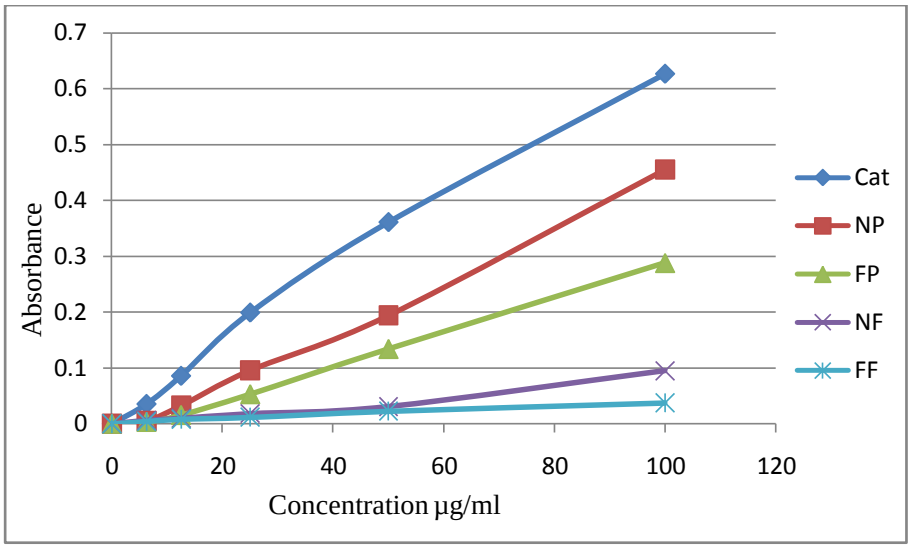

Figure 1: Total antioxidant capacity of normal and formalin treated mango peel and flesh.

3.2 Ferric reducing power assay

The reductive capabilities of crude ethanolic extract of *M. indica* of both normal and formalin treated peel and flesh are shown in the figure 2. The result demonstrated that mango peel exhibit appreciable reducing activity. Of the peel, normal mango peel (NP) shows more activity as compared to the formalin treated mango peel (FP).

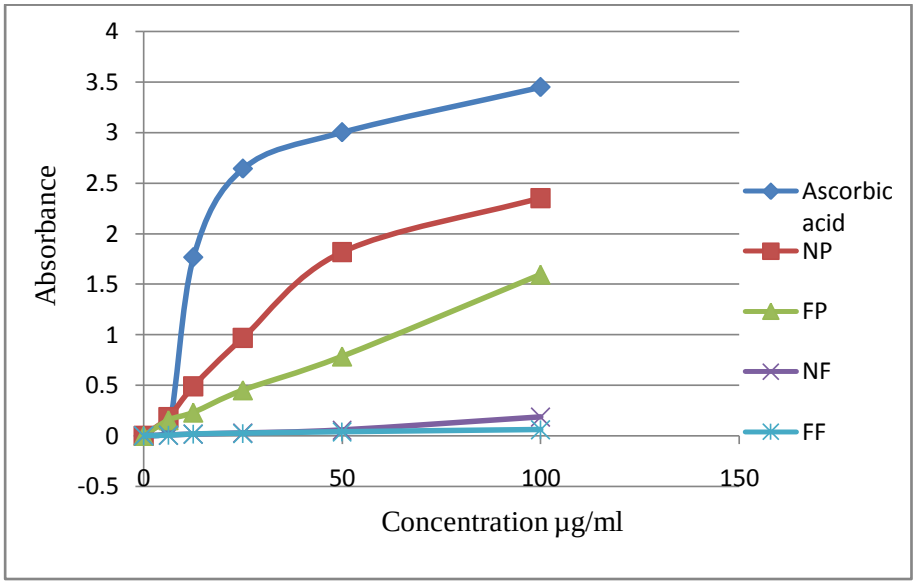

Figure 2: Reducing power capacity of the crude ethanolic extract of normal and formalin treated mango peel and flesh.

3.3 DPPH radical scavenging activity

The DPPH radical scavenging activity of mango were shown in Figure 3 and Table 1. The radical scavenging effects of the extracts shows that NP exhibit maximum scavenging activity which is almost equal to the standard compound BHT. The FP also exhibit significant scavenging activity. The other sample, NF exhibit sufficient scavenging activity whereas FF gives very low scavenging activity.

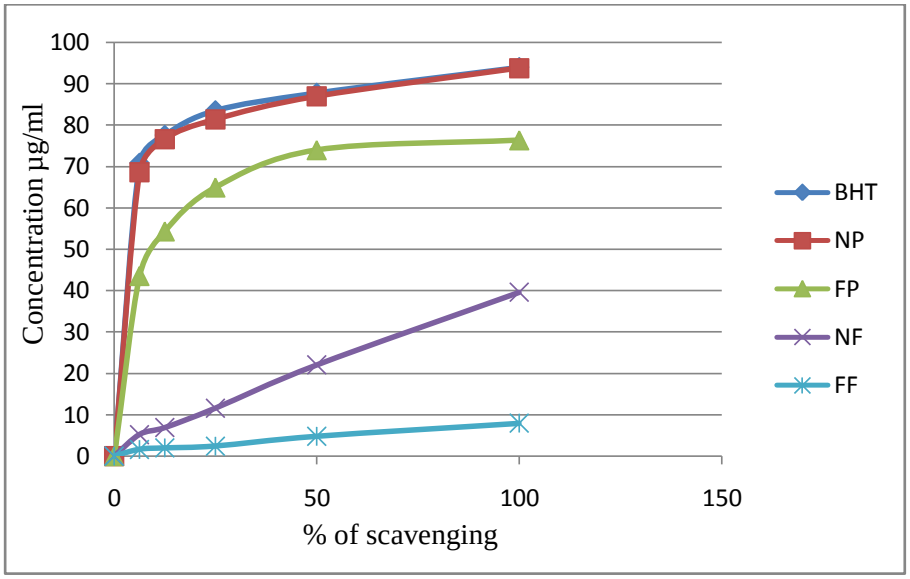

Figure 3: Free radical scavenging activity of the crude ethanolic extract of mango peel, flesh and BHT at different concentrations.

The IC_{50} of NP and FP is 4.2 and 4.9. But NF and FF have IC_{50} of 125.5 and 675.03. Low IC_{50} value indicates strong ability of the extract to act as DPPH scavenger.

Table 1: IC_{50} values (µg/ml) of crude ethanol extract of mango peels and fleshes.

Extracts	**IC_{50}(µg/ml)**
Normal mango peel (NP)	4.2
Formalin treated mango peel (FP)	4.9
Normal mango flesh (NF)	125.5
Formalin treated mango flesh (FF)	675.03
Butylated hydroxy toluene (BHT)	4.0

4. Discussion

Oxygen is indispensable for life and cells use oxygen to generate energy, while free radicals are created as a consequence of adenosine triphosphate (ATP) production by the mitochondria. These by-products are generally reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that result from the cellular redox process. These species play a dual role as both toxic and beneficial compounds [35]. At low or moderate levels, ROS and RNS exert beneficial effects on cellular responses and immune function. At high concentrations, they generate oxidative stress, a deleterious process that can damage all cell structures [36]. The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention. The antioxidant effect is mainly due to phenolic compounds [26-27, 37].

The total antioxidant activity of the extractives increased with the increasing concentration of the extracts (Figure 1). The results show that among the four ethanolic extracts, the NP has the highest antioxidant activity but treatment with formalin antioxidant activity decreases, whereas fleshes have low antioxidant activity. Literature data also shows the presence of higher antioxidant of mango peels, compared to mango flesh [38-39]. It might be suggests that formalin penetrated to the peel of mango and decreased the antioxidant potential of it. Literature suggests the penetration of formalin in to the specimen is a physical process by which the solution diffuses in to the specimen to reach the innermost layers of cells [40] and when tissues are immersed in formalin, they are rapidly penetrated [41].

The reducing capacity of ethanolic extractives increased with the increase of concentration of extracts (Figure 2). The NP exhibits maximum reducing capacity and FP exhibits moderate reducing activity. Although the other two extract, NF and FF contain reducing activity but the amount is negligible. It also indicates after formalin treatment reducing power capacity of mango decreases.

DPPH free radical scavenging is an accepted mechanism that has been used extensively to predict antioxidant activities by which antioxidants act to inhibit the free radical generation. From (Table 1 and Figure 3) it is observed that NP exhibits very high radical scavenging activity (IC_{50} is 4.2) which is almost equal to the standard, BHT (IC_{50} is 4.0). At 100 μg/ml scavenging activity of NP is 93.79%, but FP at same concentration scavenges 76.36%, where as BHT, scavenges about 93.95%. Scavenging power of NF is 39.57% and in FF scavenging potential is only 7.12% at 100 μg/ml.

5. Conclusion

This is the first reported data about comparative antioxidant activity of formalin treated mango with normal mango. The study suggests that normal mango peel contain strong antioxidant effects as compared to the mango flesh, but the use of formalin during preservation decreases that antioxidant property in mango peel as well as mango flesh, which indicated that formalin may penetrate into the mango peel and decreases antioxidant value of mango flesh. Further study is required to identify the mechanism of formalin penetration and mechanism of antagonistic antioxidant potential by formalin.

References

[1] Karthikumar P, Kishor MP, Meenakshi M. Screening of antibacterial and antioxidant activities of leaves of *Eclipta Prostrata* (L). *Scientific Research and Essay* 2007; 24:101-104.
[2] Ojo OA, Akintayo CO. Assessment of antioxidant activity of *Ficus asperifolia* aqueous extract - In vitro studies. *The Journal of Phytopharmacology* 2014; 3(1):16-21.
[3] United Nation Food and Agriculture Organization Corporate Statistical Database (UN FAOSTAT). Statistics from: Food And Agricultural Organization of United Nations: Economic And Social Department: The Statistical Division 2011.
[4] Formalin in fruits. The Daily Star, 12 June 2013. Accessed on 26 February 2014.
[5] Almost all litchis formalin-tainted. The Daily Star, 12 June, 2014. Accessed on 12 June 2014.
[6] Greg A, Wooster, Casandra M, Martinez, Paul R, Bowser. Human Health Risks Associated with Formalin Treatments Used in Aquaculture: Initial Study. *North American Journal of Aquaculture* 2005; 67:111.
[7] Wooster GA, Martinez CM, Paul RB. Human Health Risks Associated with Formalin Treatments Used in Aquaculture: Initial Study. *North American Journal of Aquaculture* 2005; 67:111-113.
[8] World Health Organization (WHO). Regional Office for Europe, Indoor Air Quality: Radon Report on a WHO Working Group. *Journal of Environmental Radioactivity* 1988; 8:73-91.
[9] International Agency for Research on Cancer (IARC). Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 88. Lyon, France 2004.
[10] Yue W, Jin XB, Pan XC, Ding J. Relationship between indoor air formaldehyde exposure and allergic asthma in adults. *Chinese Journal of Public Health* 2004; 20(8):904–906.
[11] Fan W, Zhou Y, Jin F, Du L, Jin X. The health effects of pathologists exposed to formaldehyde. *Journal of Occupational and Environmental Medicine* 2006; 23(6):466–468.
[12] Geng Y, Meng X, Li X, Lu G. Occupational damage in densified wood board producing field and its effect on workers' health. *Journal of Occupational Health* 2004; 20(8):21–22.
[13] Shi J, Zhu SX, Tong ZM, Sun DX, Yang H, Jiang RM. Epidemiological study of health effect for occupational exposure staffs to formaldehyde. *Chinese Journal of Industrial Hygiene and Occupational Medicine* 2006; 33(3):237–239.
[14] Xu SY, Yi GL, Li SH. Hygienic investigation of the effect of formaldehyde on the workers' health. *Journal of Occupational Health* 2007; 23(7):491–492.
[15] Liu SX, Guo SH. Investigation on health outcome of workers exposed to formaldehyde. *Occupational Health and Emergency Rescue* 2004; 22(2):93–94.
[16] Lu Y, Chen XJ, Yang XY, Xue ZQ. A survey of the effect on to teachers' health from formaldehyde contact. *Journal of Xinjiang Medical University* 2007; 30(3):234–237.
[17] Tang LX, Zhang YS. Health investigation on workers exposed to formaldehyde. *Journal of Occupational Health* 2003; 19(7):34–35.

[18] Yuan CH, Dong B. Health effect on anatomy teachers exposed to formaldehyde. *Chinese Journal of Clinical Anatomy* 2007; 25(3):105.
[19] Cheng Z, Li Y, Liang B, Wang C. Investigation of formaldehyde level and health of personnel in clinical pathology. *Journal of Bengbu Medical College* 2004; 29(3):266–267.
[20] Tong ZM, Zhu SX, Shi J. Effect of formaldehyde on blood component and blood biochemistry of exposed workers. *Chinese Journal of Industrial Hygiene and Occupational Medicine* 2007; 20(6):409–410.
[21] Yang WH. Hemogram of workers exposed to low concentration of formaldehyde. *American Journal of Preventive Medicine* 2007; 14(3):792.
[22] Jiang L. Menstruation of indoor formaldehyde pollution of Pingdingshan municipal office building. *Journal of Pingdingshan Institute of Technology* 2006; 15(3):38–39.
[23] Tong ZM, Shi J, Zhao JS, Yang H, Jiang RM, Kong L. Analysis on genetic toxicity of formaldehyde on occupational exposure population. *Chinese Journal of Public Health* 2006; 22(7):783–784.
[24] Yu LQ, Jiang SF, Leng SG, Zhang CZ, Yan YJ, Niu Y. Early genetic effects on workers occupationally exposed to formaldehyde. *Chin Journal of Preventive Medicine* 2005; 39(6):392–395.
[25] Zhang L, Steinmaus C, Eastmond DA, Xin XK, Smith MT. Formaldehyde exposure and leukemia: a newmeta-analysis and potential mechanisms. *Mutation Research* 2009; 681(2):150–168.
[26] Manthey JA, Veazie PP. Influences of harvest date and location on the levels of β-Carotene, ascorbic acid, total Phenols, the in Vitro antioxidant Capacity, and Phenolic Profiles of five Commercial Varieties of mango (*Mangifera indica* L). *Journal of Agricultural and Food Chemistry* 2009; 57(22):10825-10830.
[27] Sellés AJN, Castro HTV, Agüero JA, González JG, Naddeo F, Simone FD, Rastrelli L. Isolation and quantitative analysis of Phenolic antioxidants, free Sugars and Polyols from mango (*Mangifera indica* L.) Stem bark aqueous decoction used in Cuba as a nutritional Supplement. *Journal of Agricultural and Food Chemistry* 2002; 50(4):762–766.
[28] Martinez G, Delgado R, Perez G, Garrido G, Nunez Selles AJ, Leon OS. Evaluation of the *in-vitro* antioxidant activity of *Mangifera indica* L: Extract (Vimang) *Phytotherapy Research* 2000; 14:424–427.
[29] Gabino G, Deyarina G, Cheyla R, Nunez-Selles AJ, Rene D. Scavenger effect of a mango (*Mangifera indica* L.) food supplement's active ingredient on free radicals produced by human polymorpho nuclear cells and hypoxanthine–xanthine oxidase chemiluminescence systems. *Food and Chemical Toxicology* 2008; 107:1008–1014.
[30] Palmeira SMV, Gois LM, Souza LD. Extraction of phenolic compounds from mango peels. *Latin American Applied Research* 2012; 42:77-81.
[31] Umamaheswari M, Chatterjee TK. In vitro antioxidant activities of the fractions of *Coccinnia grandis* L. leaf extract. *African Journal of Traditional, Complementary and Alternative Medicine* 2008; 5:61–73.
[32] Oyaizu M. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. *Japan Journal of Nutrition* 1986; 44:307-315.
[33] Choi HY, Jhun EJ, Lim BO. Application of flow injection-chemilumineacence to the study of radical scavenging activity in plants. *Phytotherapy Research* 2000; 14:250-253.
[34] Desmarcheliar C, Repetto M, Coussio J, Liesuy S. Antioxidant and pro-oxidant activities in aqueous extract of Argentine plants. *International Journal of Pharmacognocy* 1997; 35:116-120.
[35] Valko M, Leibfritz D, Moncola J, Cronin MD. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry and Cell Biology* 2007; 39:44–84.
[36] Pham-Huy LA, He H, Pham-Huy C. Free Radicals, Antioxidants in Disease and Health. *International Journal of Biomedical Science* 2008; 4(2):89–96.
[37] Singh UP, Singh DP, Singh M. Characterization of phenolic compounds in some Indian mango cultivars. *International Journal of Food Sciences and Nutrition* 2004; 55(2):163–169.
[38] Berardini N, Fezer R, Conrad J, Beifuss U, Carle R, Schieber A. Screening of mango (*Mangifera indica* L.) Cultivars for their Contents of flavonol O- and Xanthone C-glycosides, anthocyanins, and Pectin. *Journal of Agricultural and Food Chemistry* 2005; 53(5):1563-1570.
[39] Schieber A, Ullrich W, Carle R. Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection. *Innovative Food Science and Emerging Technologies* 2000; 1:161-166.
[40] Grizzle WE, Fredenburgh JL, Myers RB. Fixation of tissues. In: Bancroft JD, Gamble M, editors. Theory and Practice of Histological Techniques. 6th ed. Philadelphia, USA: Elsevier Limited; 2008. pp. 56-63.
[41] Fox CH, Johnson FB, Whiting J, Roller PP. Formaldehyde Fixation. *Journal of Histochemistry and Cytochemistry* 1985; 33:845-853.