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Comparative phytochemical screening of normal and formalin treated mango

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Abstract
The present study was designed to evaluate phytochemical properties of mango after formalin (37% formaldehyde solution) treatment. For this purpose fresh raw mangoes are collected, washed with pure water and divided into two groups. One group was treated with formalin for 7 consecutive days and another group was kept as normal. Both group of sample are then peeled off, dried, crushed into coarse powder and extracted by ethanol under sonication bath. Then the samples are filtered and concentrated with a rotary evaporator under reduced pressure at 50 °C. Then phytochemical screening was carried out and found that treatment with formalin markedly decreased phenolic and flavonoid content of mango. Similar result was found in both peel and fleshes which indicates that formalin may penetrated into the peel and brings certain change in flesh. Protein content of mango also shows a downward movement after formalin treatment.

Keywords: Mango, phytochemical, phenolic, flavonoid, protein content

1. Introduction
Food adulteration is basically lowering the quality of food for sale either by the admixture or by the substitution of inferior substances or by the removal of some valuable nutritious ingredient. Uses of poisonous chemicals in perishable foods are evident in highest degrees which are endangering the lives of the people [1]. The Institute of Public Health (IPH) in Dhaka and the World Health Organization (WHO) in their joint study conducted in 2003 in Dhaka city and found that 96% of sweetmeats, 24% of biscuits, 54% of breads and 59% of ice creams were extensively adulterated [2]. A random survey by the Public Health Laboratory of Dhaka City Corporation in 2004 indicated that more than 76% of food items on the market were found to be adulterated and the level of food adulteration varied 70% to 90% [3]. In 2014 Transparency International Bangladesh (TIB) disclosed that at least 4.5 million people were directly affected by the consumption of tampered foods in Bangladesh [4]. Food items that are commonly adulterated include fruits, vegetables, milk, fishes, sweetmeats, rice, wheat, meat, oil, ghee, spices, egg, soft drink, juice powder, baby foods and so on [5]. Fruits are adulterated with calcium carbide, ethephon, formalin, injections of colors and sweeteners. Vegetables are adulterated with formalin and toxic dyes while fish with formalin [6]. Among them formalin is the leading adulterant which is used to adulterate various fruits and vegetables [6], milk [7], meat [8] and Sweetmeats [9].
A saturated water solution, of about 40% formaldehyde by volume or 37% by mass, is called formalin. A small amount of stabilizer, such as methanol, is usually added to suppress oxidation and polymerization [10]. Formaldehyde is an extremely reactive chemical agent and acts by forming a chemical bond with the primary amide and the amino groups of protein molecules. Thus, in vitro, it reacts with proteins, DNA & RNA and forms protein – DNA cross linkages and hence inhibits DNA synthesis [11]. Formaldehyde or its metabolites can penetrate human skin and induces contact dermatitis in humans [12]. Inhaled formaldehyde caused irritation of eyes, tearing, sneezing, coughing, chest congestion, fever, vomiting, abdominal pain and nodal tachycardia [13]. There are also reports of irritated skin, heartburn, tremor, body sores, chest pain, lethargy and loss of appetite [14-19]. Many reports indicate that chronic exposure to formaldehyde increases the chances of headache, dizziness, sleep disorders and memory loss [20-26]. Similarly, other studies showed amplified pulmonary damage with increased exposure of formalin [27-28]. Long-term exposure of formalin can decrease the number of WBC and possibly lower platelet and haemoglobin counts [29].

Mango (*Mangifera indica* L.) is a juicy stone fruit, grown in many parts of the world, particularly in tropical countries. Over 1000 mango varieties are available worldwide [30]. It is now commercially grown in more than 87 countries [31]. Mango fruit conquers the 2nd position as a tropical crop, behind only bananas in terms of production and acreage used [32]. Previous study suggests that mango peels contains various chemical constituents such as Phenolic compound (Mangiferin, Mangiferin gallate), Flavonoids (Quercetin) [33], Phenolic Acids (Gallic acid, ellagic acid, β-glucogallin and α-gallotannin) [34] and Volatile compounds (α-terpinolene, carene, myrcene, α-pinene and limonene) [35], while mango flesh also contains Phenolic compounds (Mangiferin, gallotannins) [36], Flavonoids [37] and Phenolic acids [38] but there is no study about the about the change of those chemical constituents after formalin treatment.

2. Material and Methods

2.1 Collection and preparation of samples

About 10 kg of raw mango was collected from the Chapainawabganj in Rajshahi. The mango was collected as pure state as it do not contain any foreign chemical. The mango is used for further analysis. After collection, the mango were washed thoroughly in distilled water and then 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 group of mangoes were peeled off, and peels and fleshes are oven dried at 55°C. The peels and flesh of each group are then ground into coarse powder. The samples are then extracted by ethanol under sonication bath and filtered through Whatman No.1 filter papers.

2.2 Preliminary phytochemical screening

2.2.1 Frothing test: About 0.1 gm of different fractions of the plant materials was shaken vigorously with water. Production of a persistent frothing (which remains stable on heating) indicates the presence of saponins.

2.2.2 Lead acetate test: About 5 ml of an aqueous solution of different fractions was taken in a test tube and few drops of 1% solution of lead acetate were added to the test tube. A yellow or red precipitate indicates the presence of tannins.

2.2.3 Glycoside test: Small amount of different fractions of the plant material was taken in the test tubes and was dissolved in 1 ml of water. A few drops of aqueous sodium hydroxide solution were then added to the test tubes. Development of yellow colour indicates the presence of glycosides.

2.2.4 Libermann - Burchard's test: Small amount of different fractions of the plant material was dissolved in 1 ml of chloroform. Two ml of acetic anhydride and 1 ml of concentrated sulphuric acid were added to the solution. Formation of a greenish colour which turns blue on standing indicates the presence of steroids.

2.2.5 Alkaloids test: About 0.5 gm of the extract was stirred with 5 ml of 1% hydrochloric acid on a steam bath and was filtered. One ml of the filtrate was treated with a few drops of Dragendorff's (Bismuth potassium iodide solution) reagent. Formation of orange-red precipitate indicates the presence of alkaloids.

2.2.6 Salkowski's test: 1ml of chloroform was added to 2 ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicates the presence of terpenoids.

2.2.7 Flavonoids test: 2 ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

2.2.8 Ferric chloride test: A fraction of the extracts was treated with aqueous 5% ferric chloride, formation of deep blue or black colour indicates the presence of phenols.

2.2.9 Ninhydrin test: 2ml of filtrate was treated with 2-5 drops of ninhydrin solution, placed in a boiling water bath for 1-2 minutes, formation of purple colour indicates the presence of proteins.

2.3 Total phenolic content

Total phenolic content of different extractives such as normal peel (NP), formalin treated peel (FP), normal flesh (NF) and formalin treated flesh (FF) was determined using Folin-Ciocalteu method as described by Miliauskas *et al* [39]. Gallic acid was used for the preparation of calibration curve. Volumes of 400 µL aliquots of 20, 40, 60, 80 and 100 mg/L sample solutions were added to test tubes followed by 2 mL of 10% (v/v) Folin-Ciocalteu reagent. The mixture was mixed and incubated for 5 min before addition of 2 mL 7.5% (w/v) sodium carbonate. The resulting mixture was further incubated for 1 h in dark at room temperature before absorbance was measured at 765 nm with a UV-VIS spectrophotometer. Results were expressed as mg Gallic Acid Equivalent (GAE) per 100 g dry weight of plant.

2.4 Total flavonoid content

The content of total flavonoids of different extractives, such as NP, FP, NF and FF was determined by aluminium chloride colorimetric method [40] using **(+)**-Catechin (CA) as standard. In this method, aluminum chloride formed complex with hydroxyl groups of flavonoids present in the samples. 500 µl solution of extractives and standard of different concentrations were taken and100 µl of 10% aluminium chloride solution was added into each of the test tubes. 100 µl of 1M potassium acetate solution was added into each of the test tubes and then incubated at room temperature for 30 minutes to complete the reaction. Then the absorbance of the solution was measured at 420 nm using a spectrophotometer against blank. The content of total flavonoids compounds in plant extracts in CAE was calculated by the following formula.

$$C = (c \times V)/m$$

Where,

C = total content of flavonoid compounds, mg/g of plant extract in GAE

c = the concentration of CA in mg/ml established from the calibration curve.
V = the volume of extract in ml
m = the weight of pure plant extracts in gm.

2.5 Protein content estimation

Protein in the sample was determined by Micro - Kjeldahl method [41]. At first the sample was digested by heating with concentrated sulphuric acid (H_2SO_4) in the presence of digestion mixture, Potassium Sulphate (K_2SO_4) and Cupper sulphate ($CuSO_4$). The mixture was then made alkaline with 40% Sodium Hydroxide (NaOH). Ammonium Sulphate thus formed, released ammonia, which was collected in 4% boric acid solution and titrated against standard hydrochloric acid (HCl). The percent nitrogen content of the sample was calculated by the following formula.

$$\% \text{ of nitrogen (N)} = \frac{1.4 \times (\text{ml HCl} - \text{ml blank}) \times \text{Conc of HCl}}{\text{Weight of sample (g)}}$$

Total protein was calculated by multiplying the amount of percent nitrogen with appropriate factor (6.25).
% of Protein = % N × Factor (6.25)

3. Results

3.1 Percentage of yield extract and phytochemical screening

It is found that normal mango peel (NP) contains 3.05% yield whereas formalin treated peel (FP) contains 3.12% yield. The fleshes contains slightly smaller amounts of yields, normal mango flesh (NF) contains 2.83% of yield whereas formalin treated flesh (FF) contains 2.91% of yield. The presence of saponins, tannins, glycosides, steroids and alkaloids has been shown qualitatively in the table 1.

Table 1: Phytochemical test results of different extracts of mango peel and flesh.

Phytochemical tests	NP	FP	NF	FF
Saponins	-	-	-	-
Tannins	+	+	-	-
Glycosides	+	+	++	++
Steroids	+	+	-	-
Alkaloids	-	-	-	-
Terpenes	++	+	-	-
Flavonoids	++	++	+	+
Phenolic	++++	+++	+++	++
Protein	++++	+++	+	+

Here - = Not present, + = Present in mild amount, ++ = Present in moderate amount, +++ = Present in large amount.

3.2 Total phenolic content

The content of phenolics of the extractives was calculated on the basis of the standard curve for GA (gallic acid) as shown in the figure 1. The results were expressed as mg of GAE/gm of dried extractives. Normal mango peel NP, contain highest amount of phenolic compounds followed by formalin treated peel FP, and then normal flesh NF and lastly formalin treated flesh FF. In comparison of both normal and formalin treatment, experimental data shows that there is clear reduction of phenolic compounds in both peels and flesh after formalin treatment.

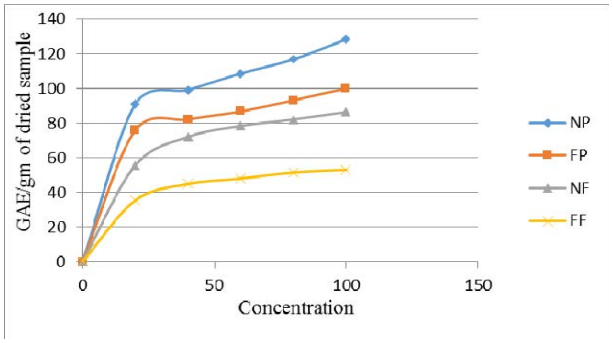

Fig 1: Phenolic content of normal and formalin treated mango. Where NP= normal mango peel, FP=formalin treated mango peel, NF=normal mango flesh, FF=formalin treated mango flesh.

3.3 Total flavonoid content

The flavonoids content of the extractives was calculated on the basis of the standard curve for catechin (CA) (figure 2), and the results were expressed as mg of CAE/gm of extractives. It is observed that normal mango peel (NP) contains highest amount of flavonoids but formalin treated peel shows reduction of flavonoids. The fleshes of mango indicates the presence of flavonoids but treatment of formalin shows the similar trend with mango peel.

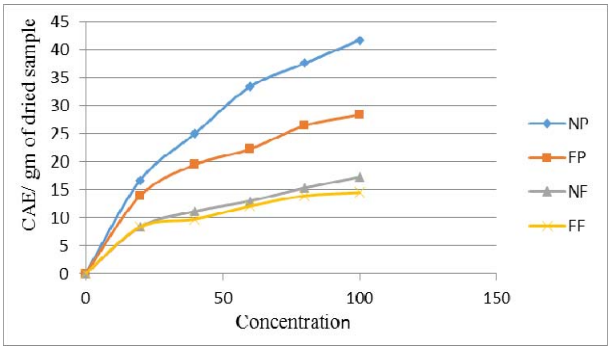

Fig 2: Flavonoid content of normal and formalin treated mango.

3.4 Protein content estimation

The protein content of the sample i.e. NP, FP, NF and FF is shown in the figure 3. The result shows that normal mango peel contain appreciable amount of protein and the amount is maximum in case of normal peel (NP) but after treatment with formalin the percentage of protein is decreased by 0.68%. Mango flesh contains very small amount of protein, indicates that it is not a useful source of protein.

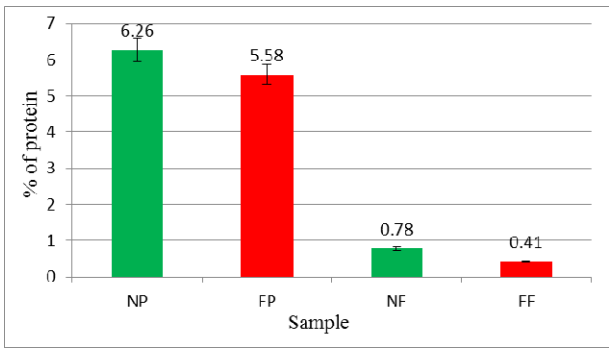

Fig 3: Protein content of mango.

4. Discussion

Phytochemical research from different parts of *M. indica* has demonstrated the presence of phenolic constituents, triterpenes, flavonoids, phytosterols, and polyphenols [42-46]. Polyphenols, the most abundant antioxidants compounds, are powerful chain breaking antioxidants that play vital role in adsorbing and neutralizing free radicals as well as quenching singlet and triplet oxygen or decomposing peroxides. Therefore, polyphenols contribute directly to antioxidative action by reducing oxidative stress [47-48]. The free radical scavenging ability of polyphenolic compounds belongs to their redox property which is due to the presence of hydroxyl groups [49]. It is observed that ethanolic extract of mango peel is a rich source of phenolic compound. The highest amount of phenolics was found in NP i.e. normal peel (128.28 ± 0.44 mg of GAE/gm of dried sample) at concentration of 100 µg/ml followed by formalin treated peel FP (99.99 ± 0.76 mg of GAE/gm of dried sample), normal mango flesh NF (86.36 ± 0.76 mg of GAE/gm of dried sample) and formalin treated flesh FF (53.03 ± 0.76mg of GAE/gm of dried sample) (Figure 1). The phenolic content is increased with the increase of concentration.

Flavonoids, another class of bioactive polyphenols, are reported to have potent antioxidant potential [50-51]. Biological systems are in equilibrium when the effects of various reactive species are offset by the activity of inherent antioxidants which may be enzymes or non-enzymatic compounds. Thus, the antioxidant activity of a system gives a measure of its protective abilities against degenerative/ oxidative reactions induced by oxidising agents [52]. More interestingly, this flavonoids has been further shown to possess antiproliferative and antitumor growth properties, thus making it a potential anticancer agent in recent years [53-55]. Among the four ethanolic extracts, the highest amount of flavonoids was found in NP (41.67 ± 1.66 mg of CAE/gm of dried extract) and lowest in FF (14.44 ± 0.96 mg of CAE/gm of dried extract) at concentration of 100 µg/ml. FP and NF contain (28.33 ± 1.67 mg of CAE/gm of dried extract) and (17.22 ± 0.96 mg of CAE/gm of dried extract) at the same concentration (Figure 2). This result demonstrated that the mango peel can act as a source for flavonoids content but treatment with formalin causes reduction of flavonoids.

Proteins are another class of bioactive compound responsible for body's defence mechanism against infection. The present study shows that mango is a source of proteins and normal mango peel (NF) contains 6.26% of protein and after treatment with formalin the peel (FP) retains 5.58% of protein (Figure 3). In addition the flesh contains negligible amount of protein.

5. Conclusion

Therefore the present study indicates that formalin decreases medicinal value of natural mango fruit and social awareness should be carried out to stop the rampant use of formalin. The decreased nutritional value of mango indicates that formalin may penetrate into the peel and contaminates into the flesh. Further study is require to determine the mechanism of formalin penetration and reduction of medicinal value.

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