



Comparative Study of Bioactive Compounds in Different Varieties of Pears in Nepal

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Article history:- Received: 5 May 2020; Revised: 7 Dec 2020; Accepted: 19 Dec 2020; Published online: 30 Dec 2020

Abstract

This study was conducted to evaluate the physicochemical parameters, perform qualitative tests (for sugars and phytochemicals), and quantitative tests (phenolics, antioxidants, anthocyanins, tannins, vitamin C) of six different varieties of pears i.e. Bartlette, Chinese pears, Chojuro, Kosui, Pharping local, and Yakumo. The juices extracted from respective pears were used for the analysis. The phenols were determined by the Folin-Ciocalteu method, antioxidants by the DPPH scavenging activity, and anthocyanins by a SO₂ bleaching technique. The Pharping local pears were found to have the highest anthocyanins (85.95±0.1 mg/l), total phenolic content (600±0.01 mg GAE/l), antioxidants (IC₅₀ value 250±0.00 mg of phenol/l) and vitamin C content (12.2±0.01 mg/100 ml) and tannins were observed to be highest in Yakumo pears (0.93±0.01 g/l). Likewise, the highest clarity i.e. 1.960±0.00 was observed in Bartlette pears and the highest acidity (2.01±0.01%) in Chojuro pears. Various sugar/carbohydrate tests like Molisch's test, Benedict's test, Barfoed test, Bial's test, Seliwanoff test, Fehling's test and Iodine test were performed for the pear varieties. All the pears gave positive results for all the sugar tests except Iodine test. The positive results for sugar/carbohydrate signifies the presence of various sugars that help for the better taste, texture, and aroma of pear. The pear varieties showed the presence of phytochemicals like flavonoids, terpenoids, catechins, cyclic glycosides, and proteins. The phytochemicals are responsible for fruit preservation and act as anti-carcinogenic components. Among the varieties of pears, Pharping local pears were observed to be most nutritional because of high antioxidants, phenols, anthocyanins, and vitamin C.

Keywords: Pears, Physicochemical parameters, Antioxidants, Phenolic content, Pharping local

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Introduction

Pear is the second most important deciduous fruit found in Nepal [1]. It is cultivated in both mid and high hills ranging from 800 to 1200 m above sea level. The pear covers 4396.5 hectares of land, a productive area of 3386 hectares with productivity of 34151 mt which yields 10.1 mt/hectares [1]. Every year, tons of pears decay during the harvesting season, because of their low consumption rate [1,2]. Pears are rich in carbohydrates, vitamin B6, vitamin A, vitamin C, sugars, iron, calcium, sodium, potassium, thiamine, water, dietary fibers, phosphorus, etc. Pears are also used as a medication to prevent the lungs' function, bones deformation, coughs, and chills, ulcers, pulmonary disease, improving immunity, etc. [1,2]. Usually, there are two types of pear grown in Nepal, i.e. European and Asian. Asian pears, which are also called Apple pears, Salad pears, Nashi, Oriental, Chinese or Japanese pears, are a large group of pears that are crispy and ready to eat as soon as they are harvested. Kosui, Chojuro, Yakumo, Hosui, Pharping local, etc are the varieties of Asian pears. European pears are harvested when they are hard and green, and stored at room temperature for the ripening process; so, they are

sweeter. The pears like Bartlette, Comice, D' Anjou are the varieties of European pears [3,4].

Pears are rich in sugars like fructose, sucrose, glucose, sorbitols, etc. Sucrose is the source of high energy and helps in cold tolerance capability of fruits; glucose and fructose act as antioxidants. Pears are also responsible for maintaining the quality of fruits and their maturity [2]. The pear juice contains 9-15 % of soluble solids [5]. The various analyses (optical and chemical) are performed to maintain the quality of juices which are called physicochemical analysis. The acidity and pH of the fruits are responsible for color, brightness, and freshness and taste of the juices [6,7]. Polyphenols are a group of compounds that use phenol as a building block. Some phenolic compounds found are gallic acid, quercetin, flavonoids, anthocyanins, (+)-catechin, tannins, epigallocatechins, resveratrol, rutin, myricetin [8,9]. Various free radicals are generated by oxidative stress and their accumulation in the cells causes oxidative damage and degeneration leading to various complications like premature aging, cataract, heart disease, and neurodegenerative disorders [10]. The compounds such as phenolics, antioxidants, and vitamin

C are responsible for the prevention as well as damage repair caused by the free radicals. Fruits are rich in these compounds, therefore, their consumption can help with bone formation, collagen, anti-inflammatory, anti-tumors/cancerous functions, etc. [11].

This research is focused on the comparative analysis of different types of pear and can help people to understand the nutritional values of pears. The data generated from this research could be useful to compare different characteristics of pear available in different origins. This research provides information about the phytochemicals, nutritional values, and sugar concentration in pears grown in Nepal. The nutritional profiling of pears could be helpful for its product commercialization in the international market.

Materials and Methods

Collection of samples

Six varieties of fully ripen pears (Bartlette, Chinese, Chojuro, Kosui, Pharping local, and Yakumo) were collected from the Warm Temperate Horticulture Centre, Kirtipur, Nepal in August, 2019.

Physicochemical analysis

pH, TSS, acidity, clarity, moisture, and ash content were determined. pH was determined using a pH meter. TSS was measured using a Brix refractometer. Clarity was determined by taking the absorbance of pear juice at 660 nm using a spectrophotometer [11,12,13].

Moisture content

For moisture content, the sample was ground and 10 gm of the sample was kept on the petri dish. The initial weight of the petri dish was noted. The plates were further placed in the oven at 105°F and the decrease in weight was noted every hour till it decreases to ± 5 mg. Before weighing, the plate was kept on a desiccator [12]. The moisture content was calculated as:

$$\text{Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \%$$

Ash content

Silica crucible was washed and dried in a hot air oven at 150 degrees for 30 minutes. The samples were ground and 10 gram was weighed. The sample was burned over a low flame furnace and was transferred to the temperature-controlled muffle furnace using long tongs. The temperature of muffle furnace was kept at 500 degrees and was left for 3-4 hrs to cool. The crucible was left to cool and was weighed [12]. The ash content was calculated as:

$$\text{Total Ash} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100 \%$$

Where, the weight of ash = Wt. of the crucible with ash - wt. of the crucible

Vitamin C content

The dye solution (mixture of sodium salt of 2, 6-dichlorophenol-indophenol dye and sodium bicarbonate) was standardized by titrating ascorbic acid with the dye solution until the appearance of pink color persists for 10-15 seconds. The dye factor i.e. mg of ascorbic acid per ml of dye was calculated using the following formula:

$$\text{Dye Factor} = 0.25 \times 10 / \text{Titre}$$

For titration, 10-20 ml sample was titrated against dye till the appearance of pink color persists for 15 seconds. The reading in the burette was noted.

Ascorbic acid (%) =

$$\frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up}}{\text{Weight of sample} \times \text{Volume of the sample taken}} \times 100$$

Metaphosphoric acid (HPO₃) acetic acid solution (3%) was prepared by dissolving 15 g metaphosphoric acid in 450 ml water, and 40 ml glacial acetic acid. The standard for Ascorbic acid was prepared by dissolving 0.05 gm of L-ascorbic to 250 ml with the metaphosphoric acetic acid solution.

For the preparation of dye solution, 0.05 g of the sodium salt of 2, 6-dichlorophenol-indophenol dye, and 0.04 g sodium bicarbonate were dissolved in 200 ml water. It was then filtered and stored in a dark-colored bottle at a refrigerated condition [13].

Qualitative tests for sugars/carbohydrates in pear juice

The various tests for sugars (Molisch's test, Benedict's test, Barfoed's test, Seliwanoff test, Fehling's test, Bial's test, and Iodine test), protein test and phytochemicals (Catechins, Flavonoids, Cyclic glycosides, Terpenoids) were performed for pear juices.

Molisch's test

Initially, 2 ml of the sample was taken, and 2-3 drops of Molisch's reagent was added. After some time, purple ring formation was observed which gives a positive test indicating the presence of all types of sugars like monosaccharides, disaccharides, and polysaccharides [12].

Benedict's test

One ml of the sample was taken initially, and 2-3 drops of benedict's reagents were added and placed in a water bath and boiled. After some time, the color of the precipitate was observed; the presence of red precipitate

gives a positive test for Benedict's test. It indicates a positive test for reducing sugars like glucose [12].

Barfoed's test

Initially, 1 ml of the sample was taken, and 2 ml of Barfoed's reagent was added. It was left to boil in the water bath and the color change was observed. Brick red color appearance indicates the positive test for reducing sugars [12].

Seliwanoff test

First of all, in 1 ml of the test solution, 2 ml of Seliwanoff reagent was mixed and kept in a water bath for 1 minute. The appearance of deep color gives a positive test for Keto sugars i.e. fructose and sucrose [14].

Fehling's test

First of all, 1 ml of the sample was taken, and 1 ml of Fehling's reagent was added. It was left in a boiling water bath. The precipitation of red color is an indication of a positive test for sugars like glucose and fructose [14].

Bial's test

Bial's reagent was added initially to the test sample. It was then kept in boiling water for some time. The appearance of blue-green color indicates positive for Ribose sugars [12].

Iodine test

This test was carried out by taking 1 ml of the sample in which 4-5 drops of iodine were added. Blue color indicates a positive test for complex sugars like Starch in a sample [12].

Phytochemicals in pear juice

Flavonoids

One ml test sample was tested with Mg metal and 5-6 drops of conc. HCl. The change in color was observed. Red color denotes flavonoids, Orange stands for flavones, and Violet indicates flavonones [14].

Terpenoids

In the mixture of 2 ml chloroform and 3 ml conc. Sulfuric acid, the sample was added and heated for 2 minutes. A grayish-reddish brown color was observed which indicates a positive test for terpenoids [14].

Cyclic glycosides

The sample was mixed with 2 ml of chloroform. Sulfuric acid was added and shake gently. The brown ring on the interface indicates the presence of cyclic glycosides [14].

Protein test

The sample was boiled with 2 ml of 0.25 % w/v of ninhydrin solution. The presence of violet-blue color is the positive test for protein [14].

Catechin test

The sample was mixed with the FeCl₃ solution. The olive green color gives the test positive for the Catechins [14].

Total Phenolic Content (TPC)

The aliquots of 1 ml gallic acid of various concentrations in methanol were added. Then, 5 ml of 10 % Folin-Ciocalteu reagent and 4 ml of 7% Na₂CO₃ were mixed up making the final volume 10 ml. The absorbance was measured at 760 nm against a blank (FC reagent+ Na₂CO₃) and the graph of the standard was plotted using the data of absorbance versus concentration (µg/ml) [13]. For determining total phenolic content in sample, 20 µl juice sample was taken and the further procedure was carried out as for the calibration solutions. The absorbance was taken and the levels of phenolic content were determined using the standard graph as Gallic Acid Equivalents (GAE) [16].

Antioxidant Content (AOC)

The stock solutions of the sample were prepared by diluting 5 ml sample(pear juice) and 10 ml of 13.5% ethanol. Diluted samples of pear juice i.e. 50, 100, 150, 200 and 250 µl were mixed with DPPH maintaining final volume 3 ml and left for 30 minutes in dark and absorbance was measured at 517 nm. The volume of wine in the diluted solutions needed to decrease the initial DPPH concentration by 50% together with the amount of phenol in mg/l was calculated. The results were used to obtain the IC₅₀ values in mg of phenol/l. The % inhibition was calculated as:

$$\text{Inhibition (\%)} = \frac{(A_c - A_s)}{A_c} \times 100$$

Where, A_c = absorbance of the control (100 µl of MeOH instead of the sample)

A_s = absorbance of the sample

The percent inhibition was plotted against volumes of wine using Microsoft Excel and the volume needed to decrease DPPH concentration by 50% was calculated from the graph. The volume of sample (diluted) that is required for decrement in the initial DPPH concentration by 50 % together with the amount of phenol in mg/l was used for attaining the value of IC₅₀ in mg of phenol/l [15].

Total Tannin Content (TTC)

200 µl of pear juice, 300 µl conc. HCl and 100 µl of distilled water were mixed in two different test tubes. The first test tube was incubated at 100°C for 30 min, whereas in the second sample, 50 µl alcohol was added and the absorbance of both samples was taken at 470 nm. The absorbance of the two samples was differentiated and represented as ΔA520 [17].

Table 1: Physicochemical parameters of pears

S.N.	Name of sample	Physicochemical parameters					
		Acidity (%)	pH	Clarity	°Bx	Moisture Content (%)	Ash content (%)
1.	Bartlette	0.134±0.01	4.13±0.01	1.960±0.00	10±0.00	16.68±0.00	0.6±0.01
2.	Chinese	0.67±0.02	4.77±0.01	2.058±0.00	11±0.00	16.97±0.02	0.21±0.00
3.	Chojuro	2.01±0.01	5.23±0.01	2.159±0.00	8±0.00	17.42±0.01	0.25±0.01
4.	Kosui	0.73±0.01	5.06±0.03	2.303±0.01	8±0.00	16.32±0.02	1.5±0.04
5.	Pharping local	0.87±0.01	4.38±0.01	2.454±0.00	7±0.00	15.85±0.00	1.3±0.01
6.	Yakumo	0.67±0.01	5.05±0.00	2.301±0.02	9±0.00	14.84±0.015	1.5±0.02

All the values (n=3) were expressed as mean ± standard deviation and found to be statistically significant (p < 0.05)

Table 2: Various sugar tests in Pear varieties

S.N.	Name of sample	Molisch's Test	Iodine test	Benedict's test	Barfoed test	Bial's test	Seliwanoff Test	Fehling's test
1.	Bartlette	+++	-	+	++	+	++	+
2.	Chinese	+++	-	+	++	+	+++	+
3.	Chojuro	+++	-	++	+++	+	+++	++
4.	Kosui	+++	-	++	+++	+	+++	++
5.	Pharping local	+++	-	++	+++	+	++	++
6.	Yakumo	+++	-	+	++	+	++	+

Note: (-) denote absence, (+) denote trace, (++) denote moderate and (+++) denote high amount of sugars. The comparison were done on the basis of colour intensities

$$\Delta A_{520} = 1.1 \times \Delta A_{470} \text{ and}$$

$$\Delta A_{520} = 1.54 \times \Delta A_{470}$$

The lowest ΔA_{520} value was chosen for the estimation of total tannin content and was represented as g/l of juice.

It is calculated as [17]:

$$TTC = 15.7 \times \text{lowest } \Delta A_{520}$$

Total Anthocyanin Content (TAC)

50 μ l pear juice, 50 μ l HCl in ethanol (0.1%), and 100 μ l aqueous HCl (20%) were mixed in two different test tubes. To the first test tube, 220 μ l of distilled water was added and the same amount of sodium bisulfite (26%) was added to the second test-tube. Then the Absorbance was measured at 520 nm against a blank (50 μ l HCl in ethanol (0.1%), 100 μ l aqueous HCl (20%), and 270 μ l distilled water). The difference was calculated and represented as ΔA_{520} [17]. The TAC as mg/l of juice was calculated as:

$$TAC = 875 \times \Delta A_{520}$$

Data analysis

The tests were performed on triplicates (n=3) and the results for quantitative tests were reported as mean ± standard deviation (S.D.). The level of significance between various parameters were determined using one way ANOVA in Microsoft Excel 2013 and the data

presented were found to be statistically significant (p < 0.05).

Results

Physicochemical parameters of pears

Total Acidity and pH were observed to be the highest in Chojuro pears i.e. 2.01±0.01 % and 5.23±0.01, respectively. Likewise, Clarity was observed to be the highest in Bartlette pears i.e. 1.960±0.00. TSS (°Bx) was observed to be highest in Chinese pears i.e. 11±0.00°Bx. Likewise, moisture content were observed to be highest in Chojuro pears i.e. 17.42±0.01 % and ash content in Kosui and Yakumo pears i.e. 1.5±0.04 and 1.5±0.02 % respectively (Table 1).

Tests for sugars/ carbohydrates

The qualitative tests were performed for the pear juices. All the varieties gave positive tests for all sugars except the Iodine test which indicated absence of starch in pears (Table 2).

Phytochemicals Screening

All the pears gave positive tests for the phytochemicals (Table 3). The highest concentration of Flavonoids, Catechins, and Cyclic glycosides were observed in Bartlette and Pharping local pears. Likewise, Terpenoids were found to be highest in Pharping local pears only.

Table 3: Qualitative analysis of Phytochemicals

S.N.	Name of sample	Flavonoids	Terpenoids	Catechins	Cyclic glycosides	Proteins
1.	Bartlette	++	+	++	++	+
2.	Chinese	+	+	+	+	+
3.	Chojuro	+	+	+	+	+
4.	Kosui	+	+	+	+	+
5.	Pharping local	++	++	++	++	+
6.	Yakumo	+	+	+	+	+

Note: (+) denote trace, (++) denote moderate and (+++) denote high amount and (-) indicates the absence of phytochemicals

Table 4: Tannins, Anthocyanins, Total Phenolic Content (TPC), Antioxidant Content (AOC), and Vitamin C in Pears

S.N.	Name of pears	Tannins (g/l)	Anthocyanins (mg/l)	TPC (mg GAE/l)	AOC (IC ₅₀ = mg of phenol/l)	Vit. C (mg/100 ml)
1.	Bartlette	0.05±0.01	45.55±0.01	501.1±0.05	299.40±0.05	6.57±0.05
2.	Chinese	0.91±0.00	55.65±0.05	273.5±0.2	549.45±0.01	6.94±0.00
3.	Chojuro	0.06±0.02	30.63±0.00	399.5±0.28	375.93±0.00	5.55±0.02
4.	Kosui	0.02±0.00	40.85±0.00	304.9±0.01	501.68±0.01	2.3±0.05
5.	Pharping local	0.04±0.00	85.95±0.1	600±0.01	250±0.00	12.2±0.01
6.	Yakumo	0.93±0.01	68.75±0.02	301.8±0.05	509.2±0.01	2.2±0.01

All the values (n=3) were expressed as mean ± standard deviation and found to be statistically significant ($p < 0.05$).

Determination of Tannins, Anthocyanins, Total Phenolic Content (TPC), Antioxidant Content (AOC) and Vitamin C in pear juice

Tannins were observed to be highest in Yakumo pears i.e. 0.93±0.01 g/l, and Anthocyanins, Total Phenolic Content, Antioxidants, and Vitamin C were observed to be highest in Pharping local pears i.e. 85.95±0.1 mg/l, 600±0.01 mg GAE/l, IC₅₀ value 250±0.00 mg of phenol/l and 12.2±0.01 mg/100 ml respectively (Table 4).

Discussion

Table 1 focuses on the physicochemical parameters like pH, TSS, clarity, moisture, and ash content. The highest clarity was found in Bartlette pears i.e. 1.960±0.00, as it showed the lowest absorbance (clarity is inversely proportional to absorbance). On similar research conducted on two different pears i.e. Shughri and Physhun pears, Shughri pears had TSS 13.58°Bx, 83.1% moisture, 3.94% ash, and 13.71°Bx, and 54.51% moisture, 1.86% ash respectively [18]. Chinese pears were found to have highest sucrose (i.e. 11±0.00°Bx) among the 6 varieties. Likewise, the moisture was observed to be the highest in Chojuro pears i.e. 17.42±0.01% and ash content in Kosui and Yakumo pears i.e. 1.5±0.04% and 1.5±0.02% respectively. Shughri and Physhun pears had higher moisture content, Ash content, and TSS than the pears in this research. Bartlette pears in earlier research was found to have the acidity 3.50-4.60% while the Bartlette pears in this research had the acidity of 0.134±0.01%. Different acidity for the same variety of pear could be because of the different climatic conditions, storage temperature, and other environmental parameters [19].

Table 2 shows the presence of sugars in different pear juice. All the pears gave positive tests for Molisch's test, Benedict's test, Barfoed test, Bial's test, Seliwanoff test, and Fehling's test but negative for Iodine test. This indicates the presence of various sugars like glucose, fructose, and sucrose and the absence of complex sugars like starch in the pears varieties. Sugar acts as a flavor enhancer, making pear sweet increase the taste, texture, color, and aroma. They also act as food preservatives. All the pears gave positive test for Molisch's test which indicates the presence of various sugars in high amount. Positive test for Benedict's test and Barfoed test indicate presence of simple sugars like glucose, fructose, galactose, etc. [11]. Those sugars were found high in Chojuro, Kosui and Pharping local pears. Positive Bial's test indicates presence of ribose sugars. The positive Seliwanoff test indicates presence of sugars like sucrose. Sucrose was found to be high in Chinese, Chojuro and Kosui pears. Positive fehling's test indicates presence of reducing sugars like glucose, fructose, lactose, etc. [14]. They were present in high amount in Chojuro, Kosui and Pharping pears. Likewise, a study was done for the analysis of various sugars in two different pear cultivars (*Pyrus communis*) [18,20]. Fructose was found to be in highest concentration, followed by other sugars like glucose, sucrose, fructose, and sorbitol.

Table 3 highlights the presence and absence of different phytochemicals in pear juice. The pears gave positive tests for phytochemicals like flavonoids, terpenoids, catechins, cyclic glycosides, and proteins. Another research conducted on pear [21] showed a high amount of catechins and flavonoids present in pears. Likewise, in

similar research [22], various phytochemicals like catechins were found.

In **Table 4**, the amount of tannins, anthocyanins, total phenolic content, antioxidants, and vitamin C in pear varieties are presented. Tannins were observed to be the highest in Yakumo pears i.e. 0.93 ± 0.01 g/l; anthocyanins, total phenolic content, antioxidants, and vitamin C were observed to be the highest in Pharping local pears i.e. 85.95 ± 0.1 mg/l, 600 ± 0.01 mg GAE/l, IC_{50} value 250 ± 0.00 mg of phenol/l, and 12.2 ± 0.01 mg/100 ml respectively. Compared to a previous similar research [23], where the level of tannin was observed to be 1.6 g/l, this study, found the level of tannin in Yakumo pear equal to 0.93 ± 0.01 g/l [24]. Anthocyanins are responsible for the red coloration in pear fruits and its development depends on heat and light. The anthocyanin level was found to be 89.5 mg/l in pears which is higher compared to pear in this research i.e. 85.95 ± 0.1 mg/l. Given that, the anthocyanin level is higher in high temperature, those pears might have grown in high temperatures as compared to the pears cultivated in Nepal [25].

Varieties of Oriental pear and Occidental pear had total phenols 78.5-83.9 mg GAE/l and high antioxidant activities. Jules d'Airolles and Abate Fetal pears showed the lowest DPPH scavenging capacity; and Cheongbae, Niitaka, and Hanareum pears were found to have high total phenolic, flavonoid contents, and higher antioxidants than other varieties [26,27]. The highest phenolic content was observed in Pharping local pears i.e. 600 ± 0.01 mg GAE/l which is higher compared to Oriental and Occidental pears. The amount of the phenolic compounds present is based on fruit source and environmental factors as well [26]. It also acts as a primary antioxidant or free radical terminators and are effective hydrogen donors [26]. The lower IC_{50} value indicates greater antioxidant activity because the value indicates the level of antioxidants essential for the reduction of free radical i.e. DPPH by 50% of initial concentration. The vitamin C content was observed to be 12.2 ± 0.01 mg/100 ml in Pharping local pears while in similar research conducted [27], it was found in the range 2.2- 6.57 mg/100 ml which is less than that of this research. This could be because of the difference in the various factors like variety, seasonal variation, environment, climate, and the difference in protocols for the determination of vitamin C.

Conclusion

Pharping local pears are found to be the most nutritious when compared to the other five varieties. Pears are the

fruits that are rich in Vitamin C, antioxidants, phenolic contents, anthocyanins etc. Along with those components, various sugars, phytochemicals like catechins, flavonoids, terpenoids, glycosides, and little protein as well. Given such richness pears in general and Pharping pears in particular are recommended as rich sources of vitamins, antioxidants, health-promoting factors.

Author's Contribution

BK performed the experiment in the lab under the supervision of AS. BK and AS contributed for original draft preparation and during revision. BK and AS contributed significantly in editing, revising and rendering the write-up. All authors have read and approved the final manuscript.

Competing Interests

The authors have no competing interests regarding the publication.

Funding

No funds have been provided for the research by any funding agency.

Acknowledgments

I would like to acknowledge my supervisor Ms. Angela Shrestha (Lecturer) for her guidance, support and supervision during the entire work. I would also like to express deepest gratitude to Mr. Sudhakar Pant (Head of Department), teachers and staffs of Microbiology department, family and friends for their help and support during this research.

Ethical Approval and Consent

Not Applicable

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