ESTIMATION OF QUERCETIN FROM *DIOSPYROS LOTUS* L. FRUIT COLLECTED FROM FOREST OF KOTLI SATTIAN, PAKISTAN

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ABSTRACT.

Within the forests of northern Pakistan, specimens of the species *Diospyros lotus* L. are likely to be found in a widespread distribution. This fruit is also known by its common name, Amlok. This fruit is very small in size, very dark in color (nearly black), and has a taste that is quite tart and astringent. For the purpose of conducting qualitative and quantitative tests of fruit for secondary metabolites such as flavonoids, quercitin, alkaloids, saponins, tannins, and total phenols, several traditional techniques were used. This fruit showed maximum positive result in quality parameters among different solvents. *Diospyros lotus* fruit resulted in higher amount of tannins among all other secondary metabolites, that might be one of the major cause of its sharp taste. It is rich in carbohydrates and contain significant amount of other phytochemicals. This fruit resulted in considerable amount of quercetin,that is an iso flavonoid. *Diospyros lotus* fruit showed significant amount of antioxidants and might reduces the heart diseases and cancer. *Diospyros lotus* showed no activity against Gram negative (E.coli) and Gram positive (Staphlococcus aureus) bacteria.

Keywords: *Diospyros lotus*, Secondary metabolites, Antioxidant activity, Antimicrobial activity.

INTRODUCTION

Northern areas of Pakistan are bestowed with large number of flora along with fruits that might have role in reducing various diseases and for the refreshing taste as well. Kotli sattian (Rawalpindi District) is blessed with variety of medicinal plants and seasonal fruits and have significantly important for their taste and nutritional value as well. Diospyros lotus L is obtained from kotli sattain area of Rawalpindi.

Diospyros lotus L. is a member of the Ebenaceae family, which is indigenous to countries ranging from the Balkan Peninsula and the Caucasus to eastern Asia and Japan. In Pakistan, it is often referred to by the names Amlok or Kala Amlok. Rripe fruits take on the appearance of dark blue-black globes around 1.5 to 2 centimeters in diameter. As fruits go through the ripening process, their color changes from green to yellow, serving as a visible indicator of their level of development (Ahmet and Kadioglu, 1999). Due to the intensity of their taste and smell, the fruits should not be consumed directly. However, as fruits reach maturity, a number of changes take place in their phytochemistry that result in an improvement in the quality of the fruits (Ayaz et al., 1997). When it has reached its full potential, the fruit will have a brown hue and will have produced two or three seeds.

It has been shown that this fruit has qualities that are anti-cancer, anti-tumor, anti-septic, and anti-fever. These fruits contain the chemical compound known as triterpenoids, which has anti-cancer, anti-allergic, and anti-inflammatory characteristics. Tocopherol was only ten times as efficient as the tannins found in persimmon (Amlok), which were twenty times as effective as

the tannins found in persimmon (Amlok) (Ebrahimzadeh et al., 2010). Tannins extracted from persimmons (Amlok) have been demonstrated to improve the overall quality of life of hypertensive rats and reduce the number of strokes that occur in these animals. According to Lillian, the ability of these persimmon tannins to fight free radicals is twenty times greater than that of vitamin E. (2007).

There was also speculation that *D. lotus* may be used as a sedative, an astringent, a laxative, a nutritive, a febrifuge, an antitussive, an antibiotic, an anticancer agent, and an agent that combats diabetes. Fruits that are used in the treatment of dry coughs, diarrhea, and high blood pressure. In light of the importance of this fruit, there has been current research conducted on the possibility that it has health advantages. (1). investigation of the quantities as well as the qualities of secondary metabolites. (2). The antibacterial properties possessed by a variety of fruit extracts (3). Analysis of the Antioxidant Capacity of Fruit Extracts.

MATERIAL AND METHODS

SAMPLE COLLECTION AND PREPARATION

For the purpose of conducting research, samples of diosypros lotus fruit were collected from a variety of locations around kotli sattian. An expert taxonomist has checked the samples to ensure that they are correctly identified, and the information has been placed into a database (voucher specimen). At first, representative samples of the fruit were air dried in the shade, followed by drying in the oven, and then grinding into a powder (40 mashes). The powder that was produced as a consequence was dried one more time in an incubator at a temperature of 37 degrees Celsius in order to remove any trace of moisture. All of the samples were stored in sterile containers at a temperature of 4 degrees Celsius, and the labels on the containers included the name of the person who took the sample as well as the location where the sample was obtained.

In order to facilitate aqueous, alcoholic, acetone, and petroleum ether extractions, respectively, fifty grams of dried fruit powder were steeped for forty-eight hours in two hundred milliliters of distilled water, fifty percent (v/v) methanols, two hundred milliliters of acetone, and two hundred milliliters of petroleum ether. The moist mixture was mixed on a regular basis at predetermined intervals. After waiting for forty-eight hours, the material was put through a muslin filter. Once again, the filtrate was filtered using separate systems comprised of Whattman filter paper No. 1. At room temperature, we gathered the evaporated and concentrated filtrates that had been collected. In preparation for subsequent usage, the concentrates were stored in a refrigerator at a temperature of 40 degrees Celsius.

Assessment of Primary and Secondary Metabolites

Macronutrients like total proteins total sugars, reducing sugars and total oils contents were determined by using AOAC method (2000). Qualitative and Quantitative analysis of secondary metabolites from various fruit samples of *D.louts* were carried out for Alkaloids, phenols, flavonoids, tannins, saponins, glycosides, plant steroids and terpenoids by using methods reported by AOAC(2000).

Analysis of Quercetin

The research on quercetin made use of a technique that was mostly derived from that which was created by Bhimanagoud et al. (1995), although with a few modifications. In order to extract quercetin, one hundred milligrams of the material were put into a volumetric flask that contained twenty milliliters of ethyl alcohol and the mixture was agitated. A volumetric flask was used to perform the sonication process on the solution for ten minutes. Before the filtrate was collected in 15 ml falcon tubes and frozen at -20 degrees Celsius for further analysis, the sample solution was filtered using Whatman filter paper No. 42.

Antioxidant Activities

DPPH radical scavenging activity assay

It was determined whether or not fruit extract has the capacity to remove the potentially dangerous 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical (Chew et al., 2008). (DPPH) + (H-A) (H-A) The scavenging process that occurs when DPPH is combined with an antioxidant is represented by the chemical equation DPPH-H + (Purple) (Yellow):

$$(DPPH) + (H\text{-}A) \quad \rightarrow \quad DPPH\text{-}H + (A)$$

(Purple) (Yellow)

Because of the presence of antioxidants, the stable free radical DPPH is changed into the less reactive DPPH-H, and as a result, its absorbance is reduced. The degree of discoloration is a reflection of the antioxidant compounds' or extracts' potential to donate hydrogen, and it also indicates how effective they are in scavenging free radicals. In three separate trials, fruit extracts were diluted to concentrations ranging from 0.2 to 0.8 mg/ml after being subjected to dilution. In this method, one milliliter of extract was mixed with one milliliter of methanol that contained 0.135 millimeters of diphenylpicrylhydrazyl. The total volume of the mixture was one milliliter. After being held in the dark for thirty minutes and sent through a vortex, the reaction that was taking place in the combination was finally finished. It was determined by using a UV/Visible spectrophotometer how much absorbance there was at 517 nm. For the purpose of determining the radical scavenging activity of fruit extract, the following equation was utilized:

DPPH Scavenging activity % = (Abs. control - Abs. sample) / (Abs. control)*100

Whereas the absorbance of control = absorbance of DPPH + methanol

Absorbance of sample = absorbance of DPPH radical + sample

In this study, the scavenging activity of DPPH radicals was evaluated in extracts of both fresh and dried D. lotus fruits using a method that had been published in a previous study. To summarize, 100 l of extract or standard was vortexed after being combined with 2.9 ml of DPPH reagent (0.1 mM in methanol). At room temperature, the absorbance of the combination was measured at regular intervals of ten minutes for a total of sixty minutes. Gallic acid was used since it is a substance that is often used as an antioxidant. An ultraviolet-visible spectrophotometer was used in order to determine the absorbance of the remaining DPPH radicals at 519 nm. On each of the test solutions, a total of three different analyses were carried out. In order to calculate the DPPH radical scavenging efficiency, the following formulas were utilized:

DPPH radical scavenging activity

(%)= [$\{A_{control} - A_{sample}\}/\{A_{control}\}$]x100

Where $A_{control}$ is the absorbance of DPHH radical in methanol, A_{sample} is absorbance of DPHH radical + sample extract/standard.

Reducing capacity assessment

The concentrations of D. lotus fruit extract used were 0.01, 0.05, and 0.075 mg/ml, and they were mixed in a final volume of 2.5 ml with a 0.02M phosphate buffer with a pH of 6.6 and 1% potassium ferricyanide [K3 Fe(CN)6]. After that, the mixture was fermented at a temperature of fifty degrees Celsius after being heated.

Antibacterial activity

For the purpose of determining whether or not fruit extracts had antibacterial activities, Staphylococcus aureus (MTCC 96), Streptococcus pyogenes (MTCC 442), Escherichia coli, and Klebsiella Pneumoniae were utilized as test organisms (MTCC 109). For the purpose of cultivating and maintaining the organisms, nutrient agar and nutrient broth medium were both used.

Antibiotic assay:

The conventional method that is used while carrying out the tests was altered in several respects. In conclusion, we pipetted each extract onto a disc made of sterile paper, which was afterwards suspended from a needle used for dissecting. When calculating the overall volume, we made sure to take into consideration the discs' theoretically maximum capacity in terms of volume. After allowing the solvent to drain, discs (up to 9 plate-1) were placed on top of surface-inoculated Petri plates with a dilution of 106 of separate test strains. The plates were then incubated for 24 hours at 37 degrees Celsius. Following that, the plates were kept for a whole day in an incubator at a temperature of 37 degrees Celsius. Similar discs manufactured with a solvent control were tested for their ability to inhibit the development of bacteria, but the results were inconclusive. Antibiotic standards were applied to each plate, and they were in the shape of ciprofloxacin discs, each containing 30 mg of the antibiotic. On three distinct plates, each of the three different sets of fruit extracts were evaluated. After the incubation period was complete, the radius of the clear zone that around the disc was measured to the nearest millimeter. In order for a duplicate extract to be considered effective, the original extract must first demonstrate that it is active in at least one of the many copies of the original extract (all of which were from the same species of fruit, but distinct fruit samples within each sample) resulted in the formation of an inhibitory zone that was more than one millimeter beyond the rim of the disc. Using discs treated with extracts and discs treated with ciprofloxacin, triplicate assays were performed in order to determine the mean and standard deviation values for the area of this zone of inhibition for each treatment group. These values were then used to calculate the mean and standard deviation values for each treatment group. However, there are certain drawbacks to the disc diffusion test,

and it should not be relied upon as the only method for determining whether or not an antimicrobial agent is effective.

Antimicrobial activity

In polarity-ordered succession, several solvents such ethanol, water, n-hexane, and ethyl acetate were utilized to extract the ground sample, which consisted of 80 mashes. After shaking the samples for 24 hours and centrifuging them at 10,000 rpm for 15 minutes, we were able to successfully extract substances using ethanol at a 1:10 concentration. After collecting the supernatants in falcon tubes equipped with weights, the residue was re-extracted using various solvents. After putting each of the solvents through the same process, the extracts were put into an incubator to be dried. As part of the modified antimicrobial test, the dried extracts were dissolved in dimethylsulfoxide (DMSO).

Microorganisms Tested

The gram-negative bacteria Escherichia coli and the gram-positive bacteria Staphylococcus aureus were tested using a method called agar well diffusion. The results of this experiment were compared. After being prepared in Lauria-Broth gL-1 in individual test tubes with 108 cfu/ml, all of the inoculums were placed in a shaking incubator at 37 degrees Celsius for a period of 24 hours.

In order to determine whether or not an agent has antibacterial properties, the agar well diffusion method was used. Lauria-Bertini (LB) agar media was prepared, autoclaved at 121 degrees Celsius for fifteen minutes, refrigerated, and then plated onto petri dishes. All of this took place under a laminar flow hood. On each plate, six millimeter-wide wells were bored into the surface, and then 30 ul of inoculums were put into each well. After that, 1000 g/75 l of each sample was added to each well, and the plates were then placed in an incubator at 37 degrees Celsius for a

period of 24 hours. Following an incubation period of twenty-four hours, the inhibition zones were measured and reported in millimeters.

Statistical analysis.

The acquired data was analyzed using ANOVA to determine the means and standard deviations.

RESULTS AND DISCUSSION

Evaluation of the Fruit's Phytochemical Content, Both in Terms of Quality and Quantitative Amount

The results of an investigation on the levels of flavonoids, phenolic acids, tannins, saponins, and alkaloids that were discovered in a selection of fruit specimens are shown here (Tables 1 and 2 and Fig.1). When compared to other species, it was shown that D. lotus had a much higher content of flavonoids (34.422.89%; see Table 2 for further details). Every single one of the phytoconstituents is necessary for the maintenance of human health and the enhancement of the overall quality of fruit.

Many people believe that flavonoids, which can be found in a variety of fruits and vegetables, are the most important source of antioxidants that can be obtained from these kinds of foods. In addition, flavonoids act as a defensive mechanism for plants and animals against illnesses that are caused by microbes. Complexes that are formed between flavonoids and the proteins of the cell wall are very toxic to pathogens (Cowan, 1999).

According to the standard curve with the equation $y = 0.002x$ and the correlation coefficient of 0.98, the phenol content of D. lotus is quantitatively equivalent to that of gallic acid. To put it

another way, the growth of human and animal tissues and cells is impossible without the presence of phenols. Only two of the numerous functions that phenols perform in living organisms are those of an antioxidant and an antibacterial, both of which help protect the organism against illness. There are alkaloids present in D. lotus with a frequency of 2.53 0.25%. On the other hand, the quantity of saponins found in D. lotus was almost nonexistent (0.980.08%). Saponins provide a wide variety of advantages, one of which is a reduction in the severity of heart issues (Chen and Blumberg, 2008; Chavan et al., 2001). Tannins were found in D. lotus at a much greater concentration (41.49%0.64) compared to the other secondary metabolites that were found in the plant. The equation $y = 0.069x$ was discovered to represent the normal curve, and its R2 value was found to be 0.98. Tannins prevent microbes from attaching themselves to the plant cell wall, which is how they acquire their antibacterial characteristics. Tannins are able to attach to polysaccharides in the cell membrane, which allows them to assist the transport of proteins as well as help in the delivery of proteins..

Table.1.Qualitative estimation of phytochemicals for fruit samples

S.No	Phytochemicals	**D.lotus**		
		1	2	3
1	Alkaloids	+	+	+
2	Carbohydrates	-	-	-
3	Saponins	+	-	-
4	Flavonoids	+	+	+
5	Proteins and amino acids	+	+	+
6	Reducing Sugars	+	+	+
7	Phenols and tannins	+	+	+
8	Cardiac glycosides	-	+	+
9	Steroids			
10	Terpenoids	+	+	+
		+	+	+
11	Glycosides	+	+	+

Values are expressed in terms of Mean ± SD after triplicate analysis (n=3)

1=Water, 2=Ethanol, 3=Methanol

+ = Present, - = Absent

Table.2. Quantitative estimation (%) of Phytochemicals from *D.lotus* fruit extracts.

Sr.No	Flavonoid	Phenol	Saponin	Tannin	Alkaloid
1	34.42±2.89	16.91±0.84	0.98±0.08	41.49±0.64	2.53±0.25
2	30.52±2.51	17.41±1.2	0.90±0.05	37.05±0.61	2.45±0.22
3	36.91±2.96	16.05±0.82	0.95±0.06	40.12±0.63	2.50±0.24

Values are expressed in terms of Mean ± SD after triplicate analysis.

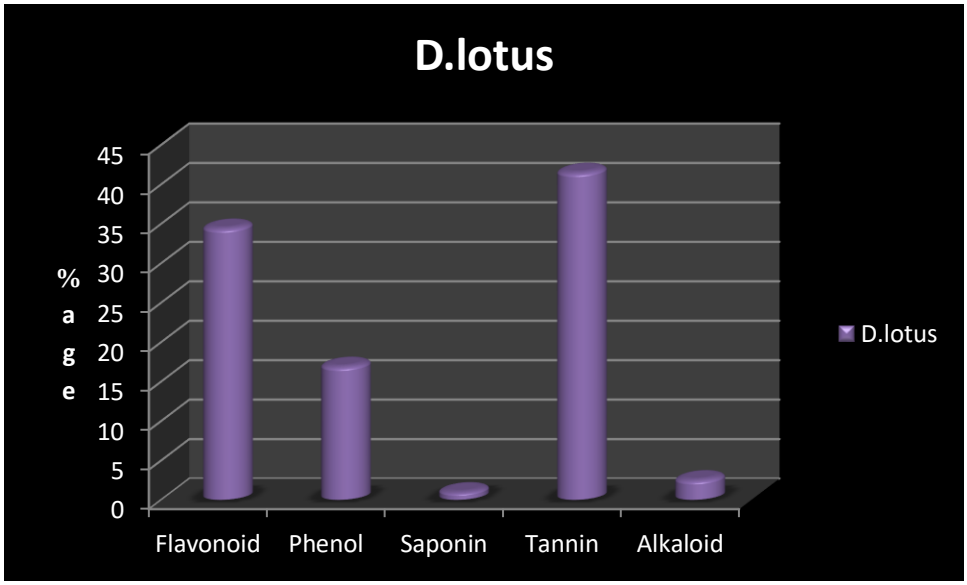

Figure 1. Comparison of various secondary metabolites obtained from fruit of *D. lotus*.

Quercetin:

D. lotus have 0.0052 ± 0.0038 with reference to Quercetin (y = 32.58x, R^2 = 0.97) as given in table 3 and Fig 2.

Table3. Estimation of quercetin from *D.lotus* fruit samples

Sr.No	Quercetin (mg/ml)
1	0.0052±0.0038
2	0.0050±0.0036
3	0.0055±0.0040

Mean values ± SD, after triplicate analysis

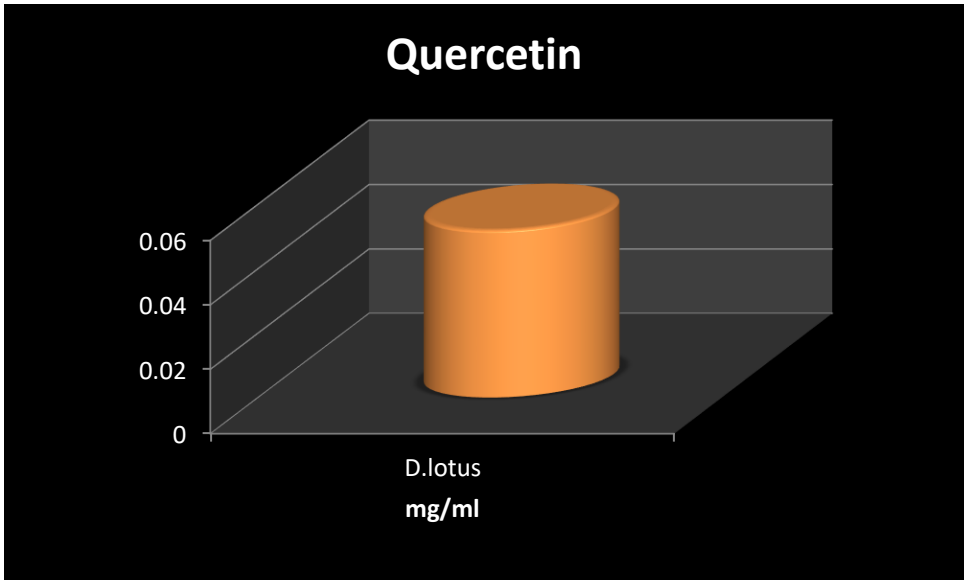

Figure 2. Analysis of quercetin from fruit extracts of D. lotus

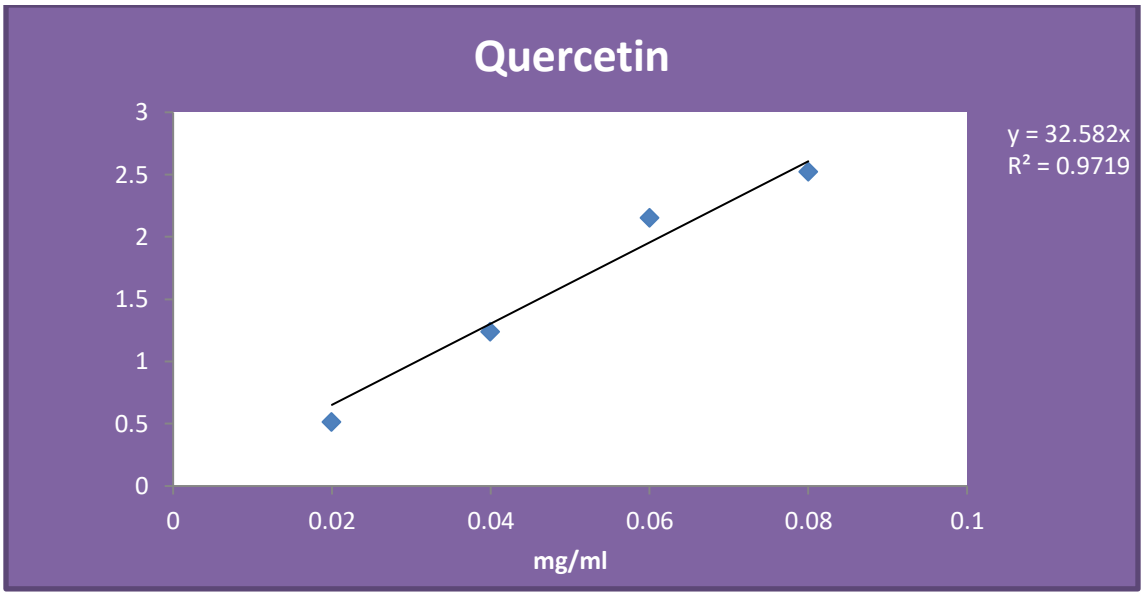

Figure 3. Graphical presentation of quercetin concentration from fruit samples

DPPH Radical Scavenging Assay

According to the findings of a great number of investigations, the 1,1,diphenyl-2-picrylhydrazyl (DPPH) test is the one that provides the most precise and trustworthy results (Ebrahmzadeh et al., 2010; Ayaz and Kadioglu,1999) It was discovered that the absorbance was best measured at a wavelength of 517 nm. Two different kinds of antioxidants, flavonoids and phenols, make connections with free radicals in order to provide protection for them. After then, the oxidative qualities of DPPH are reduced, which causes a change in its structural makeup (Durmaz and Alpaslan, 2007). The golden color takes on a more purple appearance when the DPPH levels decline. A change in hue indicates the presence of antioxidants. The antioxidant activity of D. lotus was much lower than the average (39.06% 0.73), measuring in at 22.57% 0.59. The regression equations (y = 26.39x, R2 = 0.93) and (y = 47.94x, R2 = 0.97) were used to derive the values for the inhibitory concentration half-maximum (IC50) for D. lotus (0.527 mg/ml) and

gallic acid (0.961 mg/ml), respectively. The findings were presented as a percentage in comparison to the group that served as the control (gallic acid) (Table 4 and Figs. 4-5).

To manufacture beneficial compounds, the food and pharmaceutical industries make use of antioxidants that are present in nature.

Table 4. DPPH free radical scavenging potential (517 nm) of fruits

Fruit extracts conc. (µg/ml)	***D. lotus***	**Gallic acid**
20	6.28±0.01	11.13±0.19
40	11.75±0.21	16.42±0.23
60	13.70±0.37	30.38±0.53
80	22.57±0.59	39.06±0.73

Results are obtained as triplicate analysis mean ± SD

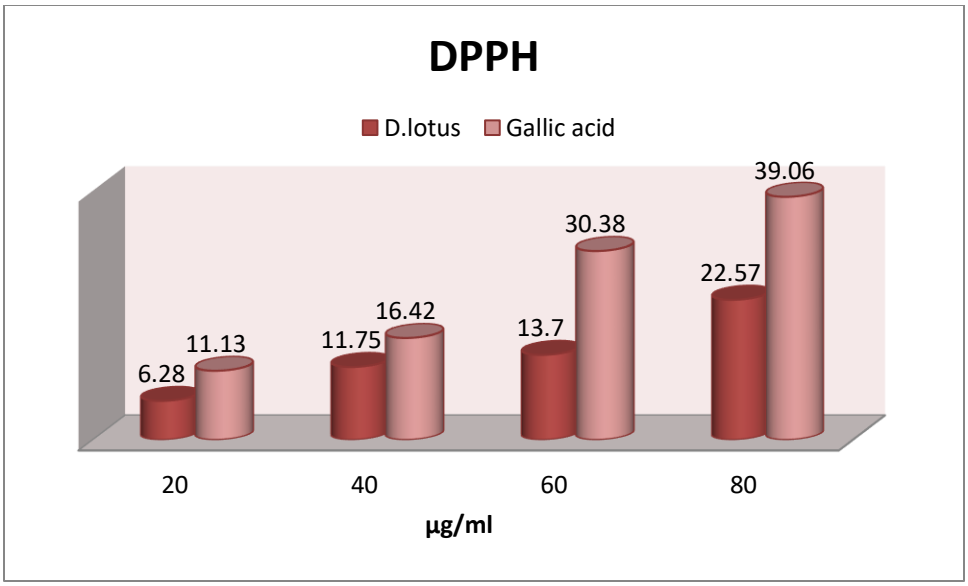

Figure 4. DPPH activity as compared to D. lotus

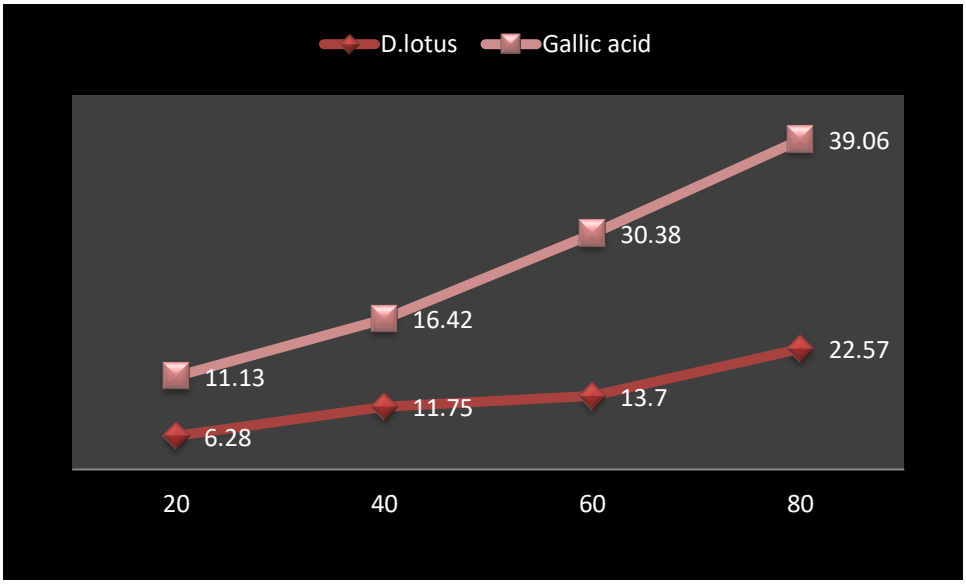

Figure 5. IC_{50} and anti radical power of different fruits and gallic acid

Antimicrobial activity of fruit extracts

In all of the extracts that were evaluated for antibacterial activity, the zone of inhibition produced by the S. aureus and E. coli bacteria was absent from the D. lotus fruit extract. in accordance with the findings in table 5.

Table 5. Antimicrobial activity of fruits against different bacteria

	D.lotus
	1 2 3 4 5
S.aureus	× × × × 32
E.coli	× × × × 42

1= Ethanol , 2= Water , 3= n-hexane , 4= Ethyl acetate, 5= Ampicillin

Flavonoids, which are essential secondary metabolites, possess anti-inflammatory effects and have the potential to cure inflammation induced by allergies, viruses, and malignancies (Cook and Samman, 1996; Bohm and Kocipani, 1994). Recent phytochemical research on the fruit extract of D. lotus reveals that it has a high concentration of beneficial flavonoids and tannins. In spite of the fact that Muhammad et al. (2008) found varying values of flavonoids (2.03 mg/g 0.01) while they were working on flavonoids of some other fruit samples, it was found that D. lotus fruit has a significant amount of flavonoids (34.42 2.89%). It was revealed that the extract of D. lotus fruit has a significant concentration of phenols (table 2). A lot of people are interested in phenols because of the antioxidant, anticancer, and antitumor qualities they possess (Muhammad, 2008). Tannins are a kind of polyphenol that dissolves in water. They have a role in the acceleration of the clotting process, the reduction of blood pressure and serum cholesterol

levels, the induction of liver necrosis, and the modification of immune responses (Hoper and Cassidy, 2006). The highest concentration of tannins was found in D. lotus at 41.49 0.64%, with values comparable to those reported by Muhammad (2008). Triterpene glycosides are the building blocks of surface-active saponins, which are produced from sterols. It is possible that the importance of saponins in diets may be linked, at least in part, to their capacity to reduce the risk of coronary heart disease. When the levels of saponins found in D.lotus fruit (0.980.08) were compared to those found in other fruits and published by Renata et al. (2011), the values found in D.lotus fruit were found to be lower.

Consumption of the antioxidant quercetin, which prevents the oxidation of low-density lipoproteins in vitro, is not associated with an increased risk of dying from coronary heart disease. According to the results of the DPPH test, the IC50 value for D. lotus was 1.894 mg/ml, which is higher than the values that were previously reported for D. lotus (Chew et al., 2008; Dragovic et al.,2007).

The use of fruit extracts from the D. lotus plant did not result in the formation of zones of inhibition against bacteria (Table 5). Diospyros species have been shown to possess inhibition zones that are effective against Escherichia coli and Staphylococcus aureus (Lillian et al., 2007). Extracts of the Diospyros plant have been proven to possess antibacterial activity, and this activity has been shown against both Gram-negative and Gram-positive bacteria.

CONCLUSION

It has been shown that consuming this fruit reduces the risk of getting stomach cancer, cardiovascular disease, and high blood pressure by a substantial amount. Phytochemicals may be a potential reason (polyphenols, flavonoids, tannins, saponins, alkaloids etc). As a result of the

high levels of both micronutrients and macronutrients that it contains, the D. lotus fruit has been found to be beneficial to human health and has the potential to be utilized as a therapy for a wide range of medical ailments.

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