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# "Evaluation of nutritional, phytochemical, antioxidant and antibacterial activity of dried plum *(Prunus domestica)*"

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**Abstract**
The nutritional, phytochemical, antioxidant and antibacterial activity of dried plum (*Prunus domestica*) were studied to understand its health benefits. The nutritional composition proved its potential as an energy source with low fat content. Protein and dietary fiber content obtained were 3.80% and 2.79% respectively. It was found to be a moderate source of minerals like magnesium, calcium, iron as well as other nutrients. The phytochemical analysis of the dried fruit revealed it to be a good source of total phenolic and flavonoids. The extract has a moderate antioxidant potential, thus confirming it as a potent electron and hydrogen donor. The GC/MS screening exhibited the presence of vitamin E, furfural, phytosterol, fatty acids, eugenol and maltol which have different therapeutic uses. In preliminary study, the extract was screened against four bacterial strains. It showed a highest zone of inhibition against *Staphylococcus epidermidis* followed by *Staphylococcus aureus* and *Proteus mirabilis.*

**Keywords:** Dried plum, Nutrition, Phytochemical, Antioxidant, Antibacterial

## 1. Introduction
Various food products have been identified as the main factors for the reduction of chronic diseases and leading a long healthy life. These products have been coined as nutraceuticals or functional foods. Antioxidant containing food products have great demand as nutraceuticals in current market. Phenolic acids and flavonoids as antioxidants are preferred due to their preventive and therapeutic nature [1, 2]. Fruits are low fat source of sodium and calories while dried fruits possess high mineral and protein content.
Plum or *Prunus domestica* (Genus: *Prunus*, Family: *Rosaceae*), a fruit deemed fit for human consumption, is known as dried plum in its dehydrated state. It prefers deciduous vegetation, with trees ranging from 12 m (39 feet) to 10m (32 feet), which flowers in April and the seed ripens during July to November. Suitable Indian terrain for the fruit is Himachal Pradesh (750 hectares) and Nilgiris (60-80 hectares) with production of plums in 2010 nationally reaching 200,000 metric tonnes. Plums possess numerous health benefits globally along with recognition in Indian medicine. These benefits range from reduction of food poisoning [3], nitrite scavenging inhibition [4], higher anti-oxidant potential, 4.4 times higher as compared to apples [5]. In addition, it acts as a scavenger against oxygen-derived free radicals such as hydroxyl and peroxyl radicals [6].
Thus, the purpose of the present study is to evaluate the nutritional, phytochemical constituents as well as secondary metabolites and also to determine the antioxidant activity along with therapeutic potential of dried plum. The objective is to identify dried plums as a potential source of natural antioxidants and to understand their nutritional and other health benefits.

## 2. Materials and Methods
### 2.1 Materials
Dried plums were purchased from local market of Haryana, India. A sample of dried plum was taken and chopped to small pieces then dried. This sample was powdered by grinding it, then further extracted and analyzed.

### 2.2 Reagents
All analytical grade chemicals, acids and solvents used in the sample preparation were

purchased from Merck Chemicals (Local supplier- Garg Chemicals, Delhi, India). All media were purchased from Hi-mediaand cultures were obtained from IMTECH (IMTECH, Chandigarh, India). All the other chemicals; TPTZ (2, 4, 6-tripyridyl-s-triazine), DPPH (2, 2-diphenyl-1- picrylhydrazyl) and ABTS (2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) were procured from Fluka (Fluka, Switzerland). HPLC grade water and solvents were procured from Rankem (RFCL ltd, India).

**2.3 Sample extraction**

50g of sample and 4 solvents, 35 ml each namely Methanol, Acetone, DCM and Ethyl acetate were added and mixed in a conical flask. The mixture was then incubated and agitated in an incubator shaker at 40 ℃ and 140 rpm for 24 h. Filtrate was collected from the resulting mixture and stored under refrigeration conditions. The collected retentate from the first filtration was again mixed with the 4 solvents and subjected to the identical process of incubation, agitation and filtration as carried out previously. The filtrates collected from this sequential procedure were mixed together and stored under room conditions to get the extract. To prevent any contamination, the samples were covered with aluminum foil with fine pores for the solvent to evaporate. The extract, obtained after double extraction with solvents, was orangish-brown in color. It was dissolved in DMSO to obtain a stock of 100 mg/ml and stored under refrigeration conditions.

**2.4 Determination of Extract Yield (EY)**

The yield of dried extract of the sample, based on dry weight was calculated from the following formula:

$$\% \text{ Yield} = (W_1 \times 100) / W_2$$

Where, $W_1$ is weight of the extract after solvent evaporation and $W_2$ is weight of the dry plant material.

**2.5 Nutritional analysis**

The total nitrogen content was determined using the Kjeldahl method [7] and obtained nitrogen was transformed into protein content by multiplying the total nitrogen by a conversion factor of 6.25. Moisture content [8] and ash content [7] were evaluated for dried plum. Fat content was determined by using petroleum ether as solvent. Crude fiber content was assessed by using the AOAC method [7]. The amount of total carbohydrates [8] and energy values [8] were also calculated. Mineral content in the dried plum was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES).

**2.6 Phytochemical analysis**

**2.6.1 Determination of crude alkaloids and saponins**

Crude alkaloid content was determined gravimetrically by Heborne method [9] and content of saponins was determined as per the method described by Obadoni and Ochuko [10].

**2.6.2 Total Phenolic content**

Total phenolics content of the sample dried plum was determined by Folin - Ciocalteu reagent method [11]. 100 µl sample was diluted with 1.15 ml of distilled water and 250 µl of Folin - Ciocalteu reagent. The mixture was mixed by vortexing it properly. Then 1.5 ml of 20% sodium carbonate was added and the mixture was incubated for 2 h at room temperature in dark. After incubation 2 ml of distilled water was added to the mixture and the absorbance of standard (gallic acid: 10–60 µg) and extract of *Prunus domestica* was measured spectrophotometrically at 765 nm against DMSO blank. The amount of total phenolics was calculated as gallic acid equivalents in mg/100 mg of dried extract.

**2.6.3 Total Flavonoid content**

Total flavonoid content was determined by colorimetric method [12]. Briefly 0.1 ml (1 mg/ml) of extract was diluted with 0.3 ml of distilled water and 0.03 ml of 5% $NaNO_2$ solution. After 5 min, 0.03 ml of 10% $AlCl_3$ was added and incubated for 5 min. Then, 0.2 ml of 1M NaOH was added and the total volume was made up to 1 ml by using distilled water. The solution was mixed properly and the absorbance was measured immediately at 510 nm. The results were expressed as catechin equivalents (CE) in mg/100 mg of dried extract.

**2.7. Assessment of Antioxidant activities**

**2.7.1 ABTS radical scavenging assay**

ABTS radical scavenging activity of the hydrophilic fractions was determined by a procedure reported by Re *et al* [13]. The ABTS solution was prepared by mixing 8 mM of ABTS salt with 3 mM of potassium persulfate in 25 ml of distilled water. The solution was held at room temperature in dark for 16 h before use. The ABTS solution was diluted with ethanol in order to obtain an absorbance between 0.8 and 0.9 at 734 nm. Fresh solution was prepared for each analysis. Antioxidant or standard solutions, 10 µl were mixed with 990 µl of diluted ABTS solution and incubated for 10 min. The absorbance at 734 nm was read. The ascorbic acid was used as a standard. The concentration of extracts required to scavenge 50% of ABTS radicals, called inhibitory concentration ($IC_{50}$) was also calculated.

**2.7.2 Ferric reducing activity power (FRAP) assay**

The FRAP reagent consisted of 10mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$ and 250 mM sodium acetate buffer (pH 3.6). FRAP reagent was freshly prepared by mixing TPTZ solution, $FeCl_3$ solution and acetate buffer in a ratio 1:1:10. A 100 µl of extract solution was mixed with 900 µl of FRAP reagent. After the mixture was incubated at 37 °C for 4 min, the absorbance at 593 nm was determined against blank. BHT was used as calibration standard. FRAP values were calculated as mg of BHTE/100 mg extract.

**2.8 GC/MS profiling of *Prunus domestica* extract**

GC/MS analysis was used to determine secondary metabolites in the sample. An Agilent 5975B mass spectrometric detector was used in the scan mode (m/z 35-1050) for dried plum. Screening of volatiles and semi volatiles were performed using the automatic RTL screener software in combination with the Agilent NIST'05 library. 1 µl of the sample from a 100 mg/ml stock was injected by split injection (1:20) at 280 ℃. The results for individual compound those quality matches > 70% is only reported (as their percentage of the total area of peaks in the total ion chromatogram).

**2.9. Antibacterial activity**

The Nutrient Agar (NA) plates were prepared for analysis and incubated at 37 ℃ for 24 h. The nutrient broth was inoculated with different bacteria (*S. epidermis*, *B. subtilis*, *P. mirabilis* and *S. aureus*) under study and left on shaker at 37 ℃ overnight. 100 µl of respective culture were pipette on

respective plates and spread over the NA plates using a sterilized spreader. Agar well diffusion method [14] was used to determine antioxidant activity against three gram-positive bacteria and one gram negative bacteria. For the agar well diffusion wells (8 mm diameter) were punched in the plates. 100 µl of the test sample was inoculated into the wells under strict aseptic conditions which are provided in laminar air flow and all the plates were incubated at 37 ℃ overnight to check the bacterial growth inhibition. Microbial growth was determined by measuring the diameter of the zone of inhibition.

## 3. Results and Discussion

### 3.1 Percentage Yield of extract

Dried plum sample was extracted by using solvent extraction method with mix solvents. The percentage yield of extract was estimated as 8.27%.

### 3.2 Nutritional Analysis

Nutritional analysis of dried plum has shown its potential health benefits (Table 1). Moisture and ash contents are very important for nutritional reporting as both of them directly affect the nutritional composition of fruits. Moisture content and ash content of dried plum were 21.5% and 20.29% respectively. The fat content of dried plum was determined by using petroleum ether and 0.20% of fat was observed in the sample; as it has low fat content, it has many health benefits.

**Table 1:** Nutritional analysis of dried plum

| Nutritional Constituents | Content |
|---|---|
| Moisture | 21.5% |
| Ash | 20.29% |
| Fat | 0.20% |
| Protein | 3.80% |
| Dietary fiber | 2.79% |
| Carbohydrates | 54.21% |
| Energy | 209.60Kcal/100g |

**Table 2:** Mineral content in dried plum

| Analyte | Concentration (ppm) |
|---|---|
| Mg | 293.2 |
| Ca | 279.0 |
| Fe | 31.32 |
| Zn | 7.649 |
| Cu | 3.482 |
| Tl | 3.291 |
| Mn | 1.933 |
| Cr | 1.790 |
| Sn | 1.183 |
| Ni | 1.076 |
| Se | 0.698 |
| Ti | 0.396 |
| As | 0.278 |
| Pb | 0.208 |

Fat is high in calories and consuming too many calories causes weight gain and obesity. A low-fat dried plum supports a steady weight and can prevent weight gain because it is lower in calories. Protein content of dried plum was found to be 3.80%. Proteins are building blocks for bones, muscles, cartilage, skin and blood as well as for enzymes, hormones and vitamins. Gravimetric method was used for the determination of dietary fibers. Dietary fiber content of dried plum was calculated to be 2.79%. Individuals with high intakes of dietary fiber appear to be at significantly lower risk for developing coronary heart disease and certain gastrointestinal diseases. Carbohydrates are among the most abundant nutrient in fruits, grains and vegetables. Carbohydrates present in the sample were calculated to be 54.21%. The energy value was calculated to be 209.60 Kcal. The mineral content showed the presence of calcium (Ca) and magnesium (Mg), in good amounts followed by other nutrients (Table 2). Moderate amounts of iron and zinc were also observed in the sample. A high Ca content can play a crucial role in providing rigidity to the skeleton besides its involvement in neuromuscular function, blood clotting and many other metabolic processes [15]. A considerably high amount of Mg can be used in building bones and in releasing energy from muscles. The mineral content also showed the presence of moderate iron content which can be utilized to metabolize proteins and helps in the production of hemoglobin and red blood cells.

### 3.3 Phytochemical analysis

Values of total phenolics, flavonoids, alkaloid and saponins were calculated (Table 3). Phenolic content (TPC) may contribute directly to anti-oxidative action. The total phenolic content of dried plum was found to be 1.05 mg GAE/100 mg extract. The total flavonoid content (TFC) of dried plum was 0.583 mg CE/100 mg extract. Percentage of alkaloids and saponins content were estimated to be 9.4% and 0.4% respectively. Alkaloids are the most efficient and therapeutically significant plant substances. These are nitrogenous organic molecules that have a pharmacological effect on humans and animals. Saponins are known as anti-nutritional factors and can reduce the uptake of certain nutrients including cholesterol and glucose at the gut through intraluminal physicochemical interaction or other yet unidentified activity [16].

**Table 3:** Phytochemical analysis of dried plum

| Phytochemicals | Content |
|---|---|
| Total phenolics | 1.05 mg GAE/100 mg extract |
| Total flavonoids | 0.583 mg CE/100 mg extract |
| Crude alkaloid | 9.4% |
| Saponin | 0.4% |

### 3.4 Antioxidant activity

#### 3.4.1 ABTS scavenging activity

The total antioxidant activity of *P. domestica* extract was evaluated in accordance with the decolorization of the ABTS to its radical cation $ABTS^{+}$ as percentage inhibition using the % radical scavenging formula. $IC_{50}$ value of the extract was calculated and it was found to be linearly increasing with the increasing concentration ranging from 1 mg/ml to 40 mg/ml. $IC_{50}$ value of the sample was estimated as 8.8 mg/ml of extract (Table 4).

#### 3.4.2 FRAP assay

The ability of the plant extracts to reduce ferric ions into ferrous ions under low pH was determined using the FRAP reagent. Antioxidants in the samples reduce ferric tripyridyl-s-triazine

complex to form blue colored complex which results in an revealed highly positive linear relation between mean FRAP values and concentration of BHT standard. FRAP reducing activity was calculated as 0.784 mg BHTE/100 mg extract (Table 4).

**Table 4:** Antioxidant activity of dried plum

| Antioxidant activity | Content |
|---|---|
| $IC_{50}$ | 8.8 mg/ml |
| FRAP reducing activity | 0.784 mg BHTE/100 mg extract |

### 3.5 Identification of Compounds by GC/MS

GC/MS screening method revealed the presence of furfural, fatty acids, vitamin E, maltol, eugenol and phytosterol (Table 5) which have different therapeutic uses. Furfural is used as a raw material in production of drug 'atropine' and has many pharmaceutical uses. These furfural derivatives have been reported for their strong bactericidal potential rather for their broad antibacterial spectrum [17]. Oleic acid is associated with decreased low density lipoprotein cholesterol and possibly increased high density lipoprotein cholesterol. The presence of vitamin E may be contributing to antioxidant activity as it is a fat-soluble antioxidant that protects the cell membranes from damage and may prevent LDL (low density lipoprotein) cholesterol from oxidizing and forming plaque in the arteries. Maltol can be used as flavour enhancer and eugenol is useful in killing parasites which could damage the heart, lungs or the kidneys. The obtained beta-sitosterol can be used for heart disease, for boosting the immune system and for preventing colon cancer.

**Table 5:** Secondary metabolites in dried plum by GC/MS screening

| Compound Name | Cas# | RT | % Area |
|---|---|---|---|
| Furfural | 000098-01-1 | 4.511 | 2.46 |
| Maltol | 000118-71-8 | 9.323 | 0.24 |
| 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 028564-83-2 | 10.018 | 1.56 |
| Benzofuran, 2,3-dihydro- | 000496-16-2 | 11.128 | 0.52 |
| 4-Mercaptophenol | 000637-89-8 | 11.936 | 8.20 |
| 5-Acetoxymethyl-2-furaldehyde | 010551-58-3 | 12.620 | 0.32 |
| Dianhydromannitol | 1000127-66-7 | 12.710 | 0.90 |
| Eugenol | 000097-53-0 | 13.327 | 0.29 |
| 2-Tetradecene, (E)- | 035953-53-8 | 13.719 | 0.19 |
| 1-Heptadecene | 000629-73-2 | 18.564 | 0.83 |
| 5-Octadecene, (E)- | 007206-19-1 | 16.299 | 0.57 |
| Hexadecanoic acid, methyl ester | 000112-39-0 | 19.944 | 0.71 |
| n-Hexadecanoic acid | 000057-10-3 | 20.336 | 2.03 |
| Dibutyl phthalate | 000084-74-2 | 20.392 | 0.46 |
| Dichloroacetic acid, heptadecyl ester | 1000282-98-2 | 20.605 | 0.37 |
| 10,13-Octadecadienoic acid, methyl ester | 056554-62-2 | 21.592 | 0.78 |
| 7-Octadecenoic acid, methyl ester | 057396-98-2 | 21.648 | 1.19 |
| Octadecanoic acid, methyl ester | 000112-61-8 | 21.861 | 0.27 |
| Oleic Acid | 000112-80-1 | 22.063 | 7.02 |
| Octadecanoic acid | 000057-11-4 | 22.220 | 1.53 |
| 2- Chloropropionic acid, octadecyl ester | 088104-31-8 | 22.445 | 0.49 |
| Cyclotetracosane | 000297-03-0 | 24.149 | 0.09 |
| Cyclopentadecanone, 2-hydroxy- | 004727-18-8 | 24.845 | 0.47 |
| 1-Nonadecene | 018435-45-5 | 25.024 | 0.12 |
| Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 023470-00-0 | 25.103 | 0.76 |
| Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 023470-00-0 | 25.181 | 0.20 |
| 1,2-Benzenedicarboxylic acid, diisooctyl ester | 027554-26-3 | 25.439 | 0.07 |
| Oleic acid, 3-hydroxypropyl ester | 000821-17-0 | 26.538 | 1.95 |
| Vitamin E | 000059-02-9 | 31.809 | 0.14 |
| Beta-Sitosterol | 000083-46-5 | 35.645 | 0.78 |

### 3.6 Antibacterial activity

Antibacterial activity of dried plum was determined against four bacterial pathogens namely *Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis and Proteus mirabilis*. Agar well diffusion method was used to determine the bioactivity by measuring the zone of inhibition. Dried plum was found to be inhibiting *Staphylococcus epidermis*, *Proteus mirabilis* and *Staphylococcus aureus*. Their antibacterial activity can be attributed to the phenolics present in the sample extracts. The sample which had higher phenolic content was found to be better in inhibiting the growth of bacteria; hence, giving a zone of clearance of greater diameter. It showed the highest zone of inhibition of 24 mm against *S. epidermidis* followed by *S. aureus* (16 mm) and *P. mirabilis* (15 mm) at a concentration of 500 mg/ml (Table 6).

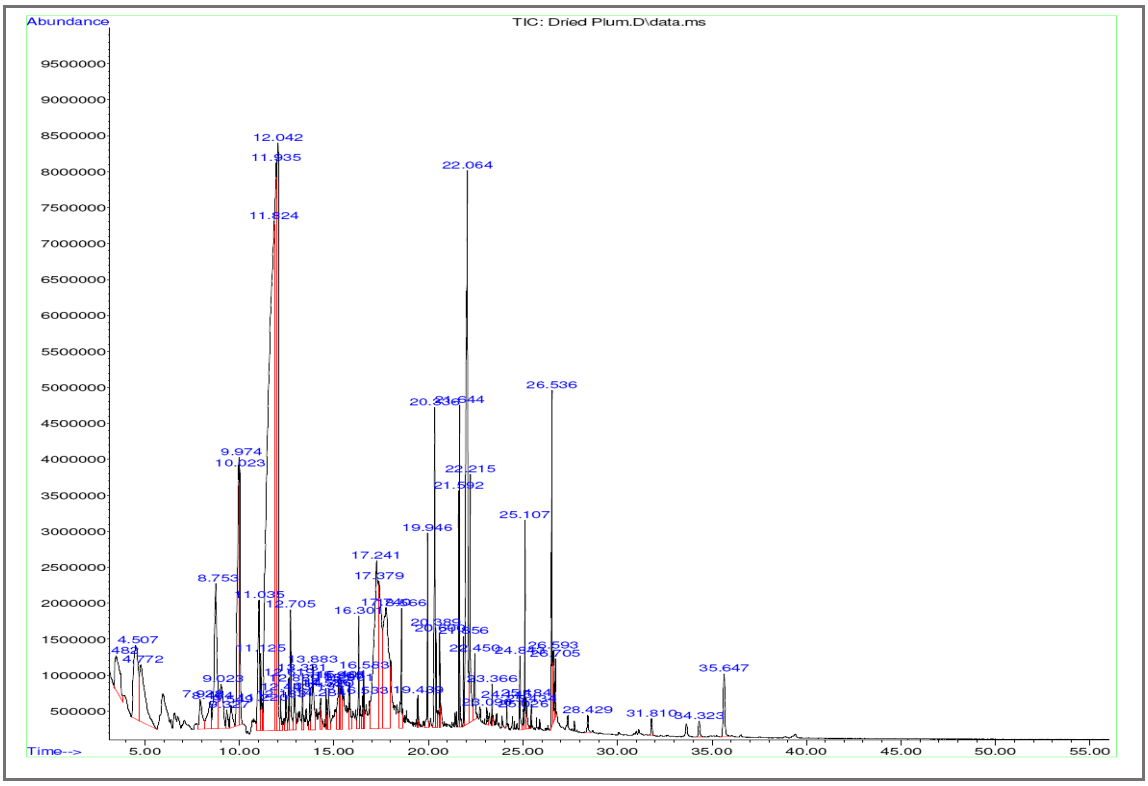


**Fig 1:** GC/MS chromatogram of mixed solvent extract of dried plum

**Table 6:** Antibacterial activity of dried plum (500 mg/ml)

| Bacterial strains | Diameter of zone of inhibition (mm) |
|---|---|
| **Gram positive** | |
| *Staphylococcus aureus* | 16 |
| *Staphylococcus epidermidis* | 24 |
| **Gram negative** | |
| *Proteus mirabilis* | 19 |

## 4. Conclusion

Dried plum was analyzed for nutritional, phytochemical, antioxidant and antibacterial activity for its potential to be used as a functional food. On the basis of the results, this dried fruit can be concluded to be a rich source of numerous bioactive compounds which collectively offer opportunities to develop value added products and other food applications to provide health benefits. The obtained compounds have potent antibacterial and antioxidant properties along with therapeutic potential which can play an important role in drug development and health supplement.

## 5. Acknowledgement

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