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Kriti Sharma
Food Processing Laboratory, University School of Biotechnology, GGS Indraprastha University, Dwarka-110078, India.

Vani Pasricha
Food Processing Laboratory, University School of Biotechnology, GGS Indraprastha University, Dwarka-110078, India.

Gouri Satpathy
Food Processing Laboratory, University School of Biotechnology, GGS Indraprastha University, Dwarka-110078, India.

Rajinder K. Gupta
Food Processing Laboratory, University School of Biotechnology, GGS Indraprastha University, Dwarka-110078, India.

Correspondence:
Rajinder K. Gupta
Food Processing Laboratory, University School of Biotechnology, GGS Indraprastha University, Dwarka-110078, India.
Email: rkg67ap@yahoo.com,
Tel: 011-25302321,
Fax: 25302305

Evaluation of phytochemical and antioxidant activity of raw *Pyrus communis* (l), an underexploited fruit

Kriti Sharma, Vani Pasricha, Gouri Satpathy, Rajinder K. Gupta

Abstract
The edible, raw (whole) *Pyrus communis* (L) (Rosaceae family) fruit was investigated for its phytochemical and antioxidant potential. The fruit was observed to be an alternative source of phenolic compounds, natural antioxidants and secondary metabolites. All assays were carried out in the methanolic extract of the fruit. The extract was reported to contain high content of total phenols and flavonoid. Antioxidant potential was analysed by using ABTS radical scavenging and FRAP assay. GC/MS analysis showed the presence of furfuryl alcohol, oleic acid, squalene and other fatty acids known to have industrial and therapeutic applications. This study demonstrates the potential of *Pyrus communis* (L) for the development of value added products with high amount of antioxidants and health promoting factors.

Keywords: *Pyrus communis* (L), Phytochemical, Antioxidant, Antibacterial, GC-MS.

1. Introduction
Pyrus communis (L), of family Rosaceae and genus *Pyrus*, is a species of pear native to central and Eastern Europe and southwest Asia [1]. It is commonly known as European pear, common pear, bagu gosha and Nakka. The fruit is a deciduous, medium-sized tree, reaching 10-17 meters height, 1-4 centimeters in diameter with an elongated basal portion. Its leaves are alternately arranged, simple, 2-12 centimeters long, glossy green and broad oval in shape. The pulp is white, aromatic and sweet with few small brown seeds embedded in it. It is consumed fresh, canned, as juice, and dried. It is a good source of dietary fiber and vitamin C [2].
Various parts of this plant like leaves, bark, flowers and roots act as anti-inflammatory agents against diseases. The leaves are a source of bioactive compounds like arbutin, isoquercitrin, sorbitol, ursolic acid, astragalin and tannin [3]. Arbutin is a natural phenolic glucoside, used in urinary therapeutics and as a human skin whitening agent. It has also been reported to be involved in defense mechanism against bacterial invasion [4]. The flowers of common pear are used in folk medicine as components of analgesic and spasmolytic drugs [5]. The bark contains friedelin, epifriedelanol and beta-sitosterol [6]. A study by Vel Murugan Cinnasamy *et al* 2014 [7] revealed wound healing activity of various extracts of *Pyrus communis* (L) in normal rats. The aim of the present study was to evaluate the phenolic compounds, antioxidant activity and the secondary metabolites of the underutilized / raw small Indian pear in order to understand the health benefits of this fruit.

2. Materials and Methods
2.1 Sample collection and extraction
The fruits were collected from a local market, New Delhi, India and were authenticated as *Pyrus communis* (L) under reference number NISCAIR/RHMD/Consult/2014/2377-157. The fruits were washed thoroughly under tap water. They were dried in an oven at 40 °C and grounded into powdered flour and stored in a closed container at room temperature for future use. 50 g of the powdered air dried sample was then mixed with 200 ml of methanol. The mixture was incubated for 24 h and then filtered. The solvent was evaporated under vacuum and resulting extracts were stored at 4 ℃.

2.2 Phytochemical analysis
2.2.1 Crude alkaloid determination
The crude alkaloid was determined gravimetrically [8]. A 2.5 g of the sample was weighed and mixed with 100 ml of 10% acetic acid in ethanol and incubated for 4 h at room temperature. The extract was filtered and concentrated to one-fourth of original volume using a water bath.

Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The precipitate was washed with a dilute ammonium hydroxide solution and filtered again. Crude alkaloid was dried and weighed. The analysis of crude alkaloids and saponins were determined on dry weight basis (g/100 g).

2.2.2 Determination of Saponins

Saponins were determined gravimetrically as per the method described by Obadoni & Ochuko (2001) [9]. A 5 g of sample was mixed with 50 ml of 20% ethanol and heated over a hot water bath for 4 h with continuous stirring at about 55 ºC. The mixture was filtered and the residue was re-extracted again with ethanol. The extracts were cooled and evaporated to 10 ml over a water bath at about 90 °C. Then the concentrate was transferred to a separating funnel and extracted with 20 ml of diethyl ether. The aqueous layer was recovered while the ether layer was discarded. The extraction was repeated again and discarded the diethyl ether layer. A 15 ml of n-butanol was added to the concentrate. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated over a water bath and the samples were dried in the oven to a constant weight. The saponin content was calculated as a percentage.

2.2.3 Determination of Total Phenolics

The concentration of total phenols in the extract was determined using Folin Ciocalteu's reagent method [10]. A 100 µl of the sample was mixed with 250 µl of Folin Ciocalteu's reagent and allowed to stand at room temperature for 5 min. Then 1.5 ml of Sodium bicarbonate (20%) was added to the mixture and incubated at room temperature for 120 min. Absorbance was measured at 765 nm using a spectrophotometer. Gallic acid (10-100 µg mL^{-1}) was used as standard for preparation of calibration curve. The amount of total phenols was calculated as Gallic acid equivalents in µg/ mg of dried extract.

2.2.4 Determination of Total Flavonoids

The Aluminium chloride colorimetric method was used for flavonoids determination **[11]**. A 250 µl of extract was mixed with 4.5 ml distilled water and 0.3 ml $NaNO_2$ (5%) and incubated for 5 min at 25 °C. Added 0.3 ml of $AlCl_3$ (10%) and wait for 5 min to complete the reaction. After another 5 min, the reaction mixture was treated with 2 ml of 1M NaOH. Finally the reaction mixture was diluted to 10 ml with distilled water and the absorbance was measured against the blank at 510 nm. Catechin was used as standard for preparation of calibration curve.

2.3 Determination of Secondary Metabolites

Secondary metabolites were determined by using an Agilent 5975B Gas chromatography mass spectrometric detector (GC-MSD) in scan mode (m/z 35-1050). The analysis was done by injecting 1 µl of the sample extract in split mode (1:20) at 280 °C. The oven was programmed from 65 ºC (5 min) at 15 ºC /min to 180 ºC (10 min), at 5 ºC /min to 280 ºC (15 min). The MS transfer line temperature was set to 300 ºC, solvent delay was 3 min, ion source and Quadrupole temperature were 230 ºC and 150 ºC, respectively. The detected peaks were searched with NIST'05 library.

2.4 Antioxidant activity

2.4.1 ABTS radical scavenging assay

The total antioxidant capacity of the extracts was determined as ABTS (2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity [12]. ABTS radicals are generated through a chemical oxidation reaction with potassium persulfate. 10 µl of extract was mixed with 990 µl of ABTS reagent and incubated for 10 min. Absorbance was measured at 734 nm. Ethanol was used as a blank. Ascorbic acid was used as standard. The percentage of radical scavenging activity was calculated using the formula:

Scavenging activity (%) = (1-Absorbance sample/ Absorbance control) x 100

2.4.2 FRAP Assay

The FRAP (Ferric reducing antioxidant power**)** assay was performed by mixing 100 µl of extract (solution containing 0.1 mg extracts) with 900 µl of FRAP reagent [13]. Absorbance was measured at 593 nm after 4 min incubation at room temperature. Butylated hydroxyl toluene was used as standard for preparation of calibration curve. The result was calculated as mg of Butylated hydroxy toluene (BHT) equivalents/mg extract. The assay was performed in triplicates.

3. Results and Discussion

3.1 Phytochemical analysis

The study has revealed the presence of substantial amounts of phytochemical content (Table 1). The *Pyrus communis* (L) has reported the presence of high quantity of alkaloid and relatively moderate amount of saponins. The occurrence of alkaloids corresponds to spasmolytic, anti-cholinergic and anesthetic activity of the extract.

The total phenols and flavonoids content of the extract was found to be quite high (Table 1). Polyphenolics are known to function as antioxidants through a number of mechanisms, including radical scavenging by H-donation, prevention of chain inhibition by donating electrons or by binding of transition metal ion catalysts [15]. Flavonoids are reported to facilitate wound healing by preventing platelet stickiness and hence platelet aggregation [16].

Table 1: Phytochemical analysis of *Pyrus communis* (L).

Sample	Total Phenolic (µg GAE/ mg).	Total flavonoid (µg CE/mg)	Crude alkaloids (g/100 g)	Saponins (g/100 g)
Methanolic extract of dried *Pyrus Communis* (L)	14.5	10.3	6.58	0.55

3.2 Antioxidant Activity

3.2.1 ABTS Scavenging assay

The antioxidant activity of *Pyrus communis* (L) was estimated as a % radical scavenging formula. The result was based on the ability of antioxidants to decolorize the $ABTS^+$ cation. The ABTS scavenging assay is applicable for screening both

lipophilic and hydrophilic antioxidants. The IC_{50} value (15.9 mg) for extract was estimated from the Figure 1. The obtained result showed a comparison between ABTS radical scavenging activity (%) of the extract and the standard (Ascorbic acid). The results revealed excellent potential of *Pyrus communis* (L) as an antioxidant sample (Table 2).

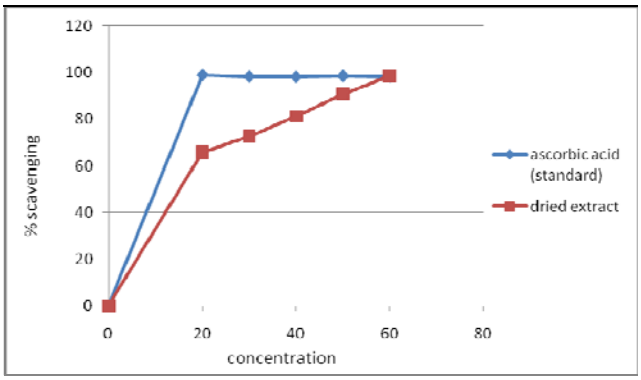

Fig 1: ABTS scavenging activity of standard and extract of *Pyrus communis* (L).

Table 2: Determination of antioxidant activity

Antioxidant potential	Antioxidant activity
ABTS	IC_{50} =15.9
FRAP	62.5 µg BE/mg of sample

3.2.2 FRAP assay

The ability of the *Pyrus communis* (L) to reduce ferric ions into ferrous ions under low pH was determined using the FRAP reagent in this assay, at acidic pH, reduction of ferric tripyridyl triazine (Fe lll TPTZ) complex to blue ferrous species can be monitored my measuring the change in absorption at 593 nm. The intensity of the colour is related to the amount of antioxidant reductants in the sample. The ferric reducing activity was observed to be 62.5 µg BHTE /mg

3.3 Screening of secondary metabolites by GC/MS

All the compounds, as identified using GC/MS analysis (Figure 2), are known to possess potent medicinal uses (Table 3). 2-Furanmethanol, found in *Pyrus communis* (L), is used as an ingredient in the manufacture of various chemical products such as foundry resins, adhesives, and wetting agents. The obtained furfural derivatives have been reported for their strong bactericidal capacity [17]. The results revealed the presence of valuable compounds having a wide variety of applications like isosorbide, Squalene, octadecanoic acid (Stearic acid) and 1-octadecdecanol (Stearyl alcohol). Isosorbide is a non-toxic diol produced from bio based feedstocks, which is biodegradable and thermally stable. It is used in medicine and has been touted as a potential biofeed stock. Squalene is used in cosmetics, and more recently as an immunologic adjuvant in vaccines. It has been proposed to be an important part of the Mediterranean diet as it may be a chemo preventive substance that protects people from cancer [18-19]. Stearic acid is mainly used in the production of detergents, soaps, and cosmetics such as shampoos and shaving cream products. Stearyl alcohol is used as an ingredient in lubricants, resins, perfumes and cosmetics and also as thickener in ointments. Few fatty acids like pentadecanoic acid, hexadecanoic acid and oleic acid (monounsaturated fatty acid, omega-9) were also reported. Oleic acid may hinder the progression of adreno leukodystrophy (ALD), a fatal disease that affects the brain and adrenal glands [20].

Table 3: GCMS profiling of methanolic extract of *Pyrus communis* (L).

Compound name	RT (min)	% Area	Cas #
2-Furanmethanol	000098-00-0	4.837	2.88
2-Cyclopentene-1,dione	000930-60-9	5.364	1.89
Propan-2-ol,1-(2methyl -4-nitroimidazol-5-ylthio)-3-(1-piperidyl)-	306326-24-9	6.014	4.94
Cyclopentanone, dimethylhydrazone	014090-60-9	8.818	1.26
1,3-Propanediamine,N-methyl-	006291-84-5	9.132	2.43
2(3H)-Furanone,5-ethyldihydro	000695-06-7	9.715	0.47
4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	028564-83-2	9.996	2.33
Pentane, 3,3-dimethyl-	000562-49-2	11.072	3.28
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	000067-47-0	11.588	9.63
1,2-Benzenediol, 3-methyl-	000488-17-5	11.925	0.65
Isosorbide	000652-67-5	12.553	1.52
Imidazolidin-4-one, 2-t-butyl-3,5- dimethyl-	1000185-71-4	13.753	0.28
Pyrimidine, 2,4,5-triamino-	003546-50-7	14.515	0.11
N1-(4-hydroxybutyl)-N3-methylguani dine acetate	1000188-13-5	15.659	0.31
d-Mannitol,1,4-anhydro-	007726-97-8	16.08	1.15
Styracitol	1000129-00-1	16.983	1.94
Squalene	000111-02-4	27.727	0.68
d-Mannitol, 1,4-anhydro-	007726-97-8	14.863	0.80
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	1000297-95-5	18.115	2.18
3-Chloropropionic acid, heptadecyl ester	1000283-05-1	19.48	0.59
Pentadecanoic acid, 14-methyl ester	005129-60-2	19.944	0.61
n-Hexadecanoic acid	000057-10-3	20.325	2.07
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	000112-63-0	21.592	0.86

Octadecanoic acid, methyl ester	000112-61-8	21.861	1.57
Oleic Acid	000112-80-1	22.063	8.54
Octadecanoic acid	000057-11-4	22.209	1.82
Oleic acid, 3-hydroxypropyl ester	000821-17-0	24.452	0.14
cis-9-Hexadecenal	056219-04-6	24.845	0.54
1-Octadecanol	000112-92-5	24.934	0.27
Hexadecanoic acid, 2-hydroxy-1- droxymethyl)ethyl ester	023470-00-0	25.181	0.89
Oleic acid, 3-hydroxypropyl ester	000821-17-0	26.538	3.40

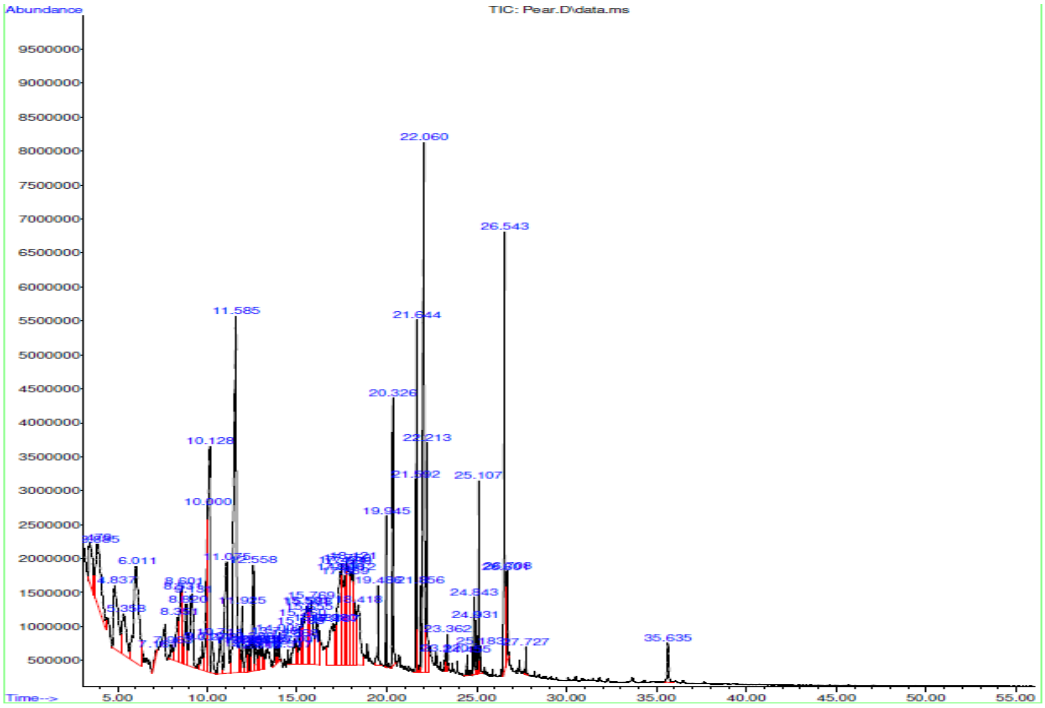

Fig 2: GCMS chromatogram of methanolic extract of *Pyrus communis* (L)

4. Conclusion

The present study has contributed to understanding the holistic and the potential application of *Pyrus communis* (L) as a source of phytochemicals like phenolics, flavonoids, alkaloids and saponins. Evaluation of secondary metabolites by GCMS revealed the presence of compounds having industrial and medicinal applications. The obtained compounds have potent antimicrobial & antioxidant properties along with therapeutic potential and may play an important role in drug development and also in the development of health supplements. The results obtained from various assays exhibited high antioxidant activity of the sample even at lower concentrations, which is in good correlation with total phenolics and flavonoids content. Based on our results, it can be concluded that the methanolic extract of *Pyrus communis* (L) is a rich source of bioactive compounds and thus offers tremendous opportunities to develop functional foods and Nutraceuticals.

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