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ISOLATION AND CHARACTERIZATION OF FATTY ACID FROM PEEL OF THE PEAR FRUIT (*PYRUS PYRIFOLIA*) AND EVALUATE ITS ANTIOXIDANT ACTIVITY

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ABSTRACT

A fatty acid was isolated and purified from pear fruit (Pyrus pyrifolia) peel extract by using column chromatography technique and evaluated its antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay and reducing power assay. The isolated compound was identified as Gadoleic acid on the basis of nuclear magnetic resonance spectroscopy and it showed the high percentage of antioxidant activity but less than the ascorbic acid. As the concentration of the compound increased the percentage of activity also increased in both the DPPH assay and reducing power assay. Thus it showed that pear is the fruit with full of phytopchemicals and having strong antioxidant activity.

INTRODUCTION

Consuming a diet high in fruits is associated with lower risk for numerous chronic diseases, including cancer and cardiovascular disease (Ness and Fowles, 1997, Block G *et al*., 1992). The overall nutritional and functional food value of fruits can be better understood by their antioxidants and phytoconstituents profile which depend on the type of fruits and their cultivation condition (Scalzo *et al*., 2005). Fruits contain a wide array of dietary phytonutrients like flavonoids, phenolic acids, carotenoids with strong antioxidant capacities (Oliviera *et al*., 2009). Among fruits, the pear is a truly wondrous hardy fruit, widely grown in the temperate regions of the world, with varied size, shape and flavors and is one of the most widely consumed fruit in the world (Jules janick, 2002). Despite the widespread consumption of pear fruits, information on the biological effects of pear fruit is very limited. Previous studies on pear fruit have focused on its chemical composition such as sugars, minerals, amino acids and phenolics (Chen *et al.,* 2007, colaric *et al*., 2006,). It has a low content of protein and lipids but rich in sugars like fructose, sorbitol, and sucrose. It has been found that the fruit contain 12.4% sugars, 0.5% protein, 0.3% lipids and 2.8% fiber (Barroca *et al*., 2006). Pear is a fruit blessed with several nutritional values, hence serve as a useful energy drink. It is being used in the treatment of various diseases from ancient time. It serves as an economical package of health care. Pear has numerous medicinal properties such as hypolipidemic, antioxidant, sedative, anti-inflammatory, anti-cancer, wound healing, anti-bacterial, anti-diabetic etc. pear provides energy to the body and boosts up immune system (Hagen, 2006, Nunes *et al*., 2008). The pear peels have much higher phytoconstituents than the pulp of the fruit (Manzoor *et al.,* 2012). However there has not been any comprehensive study on the phytoconstituents of the skin of most pears. Thus, we performed isolation and structural elucidation of chemical constituent from the fruit peel of Asian pear (*Pyrus pyrifolia*) which is one of the most highly consumed fruit in India and the peel was used to investigate the constituent contained in the pear fruit and evaluate their antioxidant activity. Recently, we reported the isolation of Gadoleic acid from the fruit peels of Asian pear.

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MATERIALS AND METHODS

Chemicals and reagents: Chloroform, methanol, acetone, ethanol, silica gel for column (BDH, 60-120 mesh), silica gel-

G for TLC, Acetic acid, Pyridine, Liquid ammonia, 2, 2-diphenyl-1-picrylhydrazyl (DPPH). All the chemicals and reagents used in the present investigation were from Merck or Sigma Aldrich.

Extraction of Sample: The pear fruit from the local market of Allahabad was purchased. Their peels were separated from the pulp. The peels were chopped and shade dried at room temperature. Then soak it into methanol for 48 hrs. After that it was filtered and distilled out. This methanol extract of peel had showed no any spot on TLC plate in any solvent. Thus, the acetate forms of this extract were made by mixing pyridine and acetic anhydride in equal amount to the extract (1ml extract+ 1ml pyridine+ 1ml acetic anhydride) heat and cool them.

Purification and isolation: Purification has been done by column chromatography. The methanolic acetate extract chromatographed over 2gm silica with a mobile phase hexane. All fractions obtained in the purification process were spotted on a silica gel TLC for analysis by using the mixture of chloroform and methanol on different polarities. The purity of fractionated compounds was visualized by heating and spraying of 1% H_2SO_4 solution. Purified compound then deacetatylated by dissolving in 1ml each chloroform and liquid ammonia and kept it for 24 hrs.

Structural analysis: NMR spectra were obtained from a unit INOVA 300 spectrometer (CDRI, Lucknow) using Tetramethyl silane (TMS) as an internal standard in $CDCl_3$.

Antioxidant activity of the purified compound

DPPH radical scavenging activity: The DPPH radical scavenging assay elucidated by Chan *et al.,* (2007) was followed. Different dilutions of the pure compounds (200,400, 600,800 µg/ml) were prepared. DPPH solution was also prepared by dissolving 4 mg of DPPH in 100 ml ethanol. Then, 2 ml of compounds from each dilution was added into the test tube containing 2 ml of DPPH solution. Control was prepared by adding 2 ml of ethanol to 2 ml of DPPH solution. Ascorbic acid was used as standard. The mixture was shaken vigorously and was left to stand in the dark for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at max of 517 nm. The scavenging activity of each compound on DPPH radical was calculated using the following equation.

Calculation: % scavenging activity = [absorbance of control-absorbance of sample / absorbance of control] × 100

Reducing power activity: Antioxidant activity by reducing power assay Yen and Duh, (1993) was followed. The reducing power of the test sample was determined by taking different concentration of the compounds (200, 400, 600, 800 µg/ml) in 1ml of water. They were mixed with 2.5ml of Phosphate buffer and 2.5ml of Potassium ferricyanide in test tubes. The mixtures were incubated for 20min at 50^0 C. At the end of the incubation 2.5ml of Tri-chloroacetic acid was added to the mixtures followed by centrifuging at 500 rpm for 10 min. The upper layer 2.5 ml was taken and mixed in 2.5 ml distilled water and 0.2 ml of ferric chloride. The absorbance was measured at 700nm. The reducing power tests were run in triplicates.

RESULTS AND DISCUSSION

The acetylated methanolic extract of peel was chromatographed over 2gm silica in Hexane. The compound was isolated in pure chloroform and the weight was 40.8mg. The structure of the isolated compound was determined on the basis of spectral data. Brown sticky compound was obtained. 1H NMR ($CDCl_3$): δ 5.02 (1H, m, H-9), 4.96 (1H, m, H-10), 2.31 (2H, t, J=7.2 Hz, H_2-2), 2.20 (2H,m, H_2-8), 2.02 (2H, m, H_2-11), 1.62 (2H, m, CH2), 1.29 (24 H, br s, 12×CH_2), 0.88 (3H, t, J=6.8 Hz, Me-20). 13 C NMR (CDCl3): δ 180.21 (C-1), 33.82 (C-2), 30.04 (C-3), 29.69 (C-4), 29.58 (C-5), 29.51 (C-6), 29.46 (C-7), 31.93 (C-8), 139.28 (C-9), 114.06 (C-10), 31.62 (C-11), 29.36 (C-12), 29.25 (C-13), 29.20 (C-14), 29.16 (C-15), 28.96 (C-16), 25.91 (C-17), 23.58 (C-18), 22.69 (C-19), 14.11 (C-20) 13C NMR spectrum revealed a carboxylic group at δ 180.21 and showed the vinylic carbon at 139.28 and 114.06. This was also supported by 1H NMR spectrum signals at 5.02 (1H, m) and δ 4.96 (1H, m).

Structure of the compound

CH_3 $(CH_2)_9$ CH=CH $(CH_2)_7$-COOH
Gadoleic acid

Table 1. DPPH free radical scavenging activity of compound

Conc.(µg/ml)	Activity %	Ascorbic acid	Activity %	Compound
200	68.87	0.061±0.0021	19.35	0.599±0.0712
400	69.89	0.060±0.0015	35.85	0.471±0.0560
600	71.42	0.056±0.0025	49.40	0.36±0.0559
800	78.06	0.043±0.0015	77.33	0.190±0.0591

Values were expressed as MEAN ± S.D. (n=3)

Table 2. Reducing power activity of compound

Conc. (µg/ml)	Ascorbic acid	Compound
200	0.545±0.0030	0.458±0.0090
400	0.604±0.0074	0.513±0.0131
600	0.634±0.0053	0.569±0.0155
800	0.695±0.0061	0.688±0.0078

Values were expressed as MEAN ± S.D. (n=3)

The result of antioxidant activity by DPPH method showed that at 200µg/ml of purified compound exhibited free radical scavenging potential (19.35%). Likewise, in 400µg/ml of compound showed (35.85%). 600ug/ml had higher free radical scavenging activity (49.40%) Whereas, 800 µg/ml of compound had (77.33%) activity. It was observed that the compound had lower antioxidant activity compared to ascorbic acid (78.06%). The result of antioxidant activity by reducing power assay as shown in table 2 it is evident that compound showed maximum absorbance at 800 µg/ml 0.688. The data showed that the compound increased their reducing ability when the concentration of compound was increased but had lowest reducing power as compared to ascorbic acid. The most utilized solvent for determination of the radical scavenging activity by DPPH is methanol. It is as a solvent used by Miller *et al*. (2000); Prakash (2001); Kim *et al*. (2002); Gupta *et al*. (2007). The concentration of the DPPH working solution in discussed methods ranges in a wide limits (Miller *et al*., 2000;

Kim *et al*., 2002; Gupta *et al.*, 2007; Silva 2004). The duration of the reaction of radical scavenging activity between DPPH solutions and sample varied from 30 min has been also showed by Kim *et al*., 2002; Kwon *et al*., 2003; Gupta *et al*., 2007. The determination of radical scavenging activity by DPPH is effectuated under different wave lengths. Prakash 2001; Ismail and Hong 2002; Kwon *et al*., 2003; Silva 2004; Gupta *et al*., 2007; Kamkar *et al*., 2010 has been used the 517 nm wave length. The literature survey indicated that Ascorbic acid was used for expression of the results as a standard (Kwon *et al*., 2003; Liebenberg 2004; Wang and Li 2007; Shikanga *et al*., 2010). The highest activity in peel rather than pulp has been showed in several fruits (jyaprakash 2001, Manzoor 2012). Many researchers have showed that the pear skin has higher activity than pulp of the fruit (Escarpa *et al*., 2001, Imeh *et al*., 2002, Oleszek *et al*., 1994, Schieber et al., 2001, Es-Safi *et al*., 2006).

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