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Total phenolic, flavonoids and tannin content of various extracts from *Pyrus communis* fruit

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ABSTRACT

The pyrus communis commonly known as Pear fruit having numerous pharmacological properties. Natural bioactive compounds like phenols, tannin and flavonoids are the important secondary metabolites in plant possess wide range biological action and this will supported with scientific studies on these metabolite from pear fruit. To maximize these agents in the extract different solvents viz. chloroform, ethyl acetate, ethanol and aqueous are used for the extraction procedure. Current study was aimed to determine the levels of total phenolic, flavonoids and tannin contents. Observations suggested that ethyl acetate and ethanol extracts has significantly high ($P<0.001$) concentration of flavonoids, phenolic and tannin contents as compared to aqueous and chloroform extracts. Therefore, ethyl acetate and ethanol extracts of pyrus communis has greater potential to produce more beneficial effects in biological system as compared to aqueous and chloroform extracts.

Keywords: *Pyrus communis*, Flavanoid, tannin and phenolic compound

INTRODUCTION

The Pear (*Pyrus communis* L.) is among the most economically important fruit tree crops of the temperate zones [1]. It belongs to family Rosaceae. It also called Common Pear- in English, in Hindi – Babbugoshaa, in Sanskrit – Amritphala and tamil- perikkai. Ancient Greek poet Homer described Pears as one of the 'gifts of God'. This prehistoric fruit has been under cultivation both in Europe and Asia for long times, also known as European Pear [2] Sand pear (Japanese and Chinese species) has been domesticated as edible fruit and cultivated in Asia for more than 3000 years [3].It has astringent, sedative activity, act as febrifuge. Its leaves and bark can be used in wound healing on account of their astringent action. [4] It is an antioxidant. It acts against reactive Oxygen species [5, 6]. The flowers of common pear are used in folk

medicine as components of analgesic and spasmolytic drugs [7]. Natural bioactive compounds like phenols and flavonoids are the important secondary metabolites in plants having intrinsic properties that affect appearance, taste, odor and oxidative stability of plant based foods. These compounds also possess biological properties like antioxidant, anti-aging, anti-carcinogen, protection from cardiovascular, immune/autoimmune diseases and brain dysfunctions viz. Parkinson's, Alzheimer's, Huntington's diseases, etc [8, 9]. Due to their large biological activities, plant secondary metabolites have been used for centuries in traditional medicine. Nowadays, they correspond to valuable compounds such as pharmaceutics, cosmetics, fine chemicals, or more recently nutraceutics. Recent surveys have established that in western countries, where chemistry is the backbone of the pharmaceutical industry, 25% of the molecules used are of natural plant origin [10].

Therapeutic potential of Pyrus communis extract is directly related to total phenolic, Tannin and flavonoids contents. These active metabolites especially from herbs are the interest subject of research, but their extraction as part of phytochemical or biological investigations presents specific challenges that must be addressed through out the solvent extraction process. Therefore, present study was aimed to investigate the levels of phenolic, flavonoids and tannin contents in different extracts prepared using chloroform, Ethyl acetate, ethanolic and aqueous solvents by spectrophotometric methods.

MATERIAL AND METHODS

Chemicals

Chloroform, ethanol, petroleum ether, ethyl acetate and sodium bi carbonate were purchased from BVN Chemicals, India. Standards of phenolic acids (gallic acid), tannin (tannic acid) and of flavonoids (rutin hydrate) were purchased from loba chemie Pvt Ltd, Mumbai. The Folin- Ciocalteu's phenol reagent and Folin denis reagentwere from s d fine chem ltd. Aluminium chloride (AlCl3) were from Indian chemicals, Bangalore, India. All other solvents and chemicals were of analytical grade.

Collection and authentication of the plant material

The fruits of pyrus communis had been collected from Madanapalle, Chittoor District, Andhra pradesh, India. The fruit was identified and authenticated by the Botanist Dr. K. Madhava Chetty, Assistant Professor, Department of botany, Sri Venkateswara University, Tirupathi.

Preparation of extracts

The collected fruits were shade dried completely. The dried fruit was then coarsely powdered and was sieved (sieve # 60) to get uniform powdered. The powdered materials were defatted with Petroleum ether by maceration for 48 hours. The marc was dried and successive extracted with solvent chloroform, ethyl acetate, 80% ethanol and aqueous by maceration process. Final compound was concentrated by vacuum drying. The traces of the solvents were removed by keeping the dried extracts in to desiccators.

Preliminary phytochemical screening

The different extracts of fruits of pyrus communis was screened for the presence of various phyto constituents like alkaloids, flavonoids, saponins, tannin, glycosides [11].

Determination of total phenolic contents in the extracts [12]

The concentration of total phenol in plant extracts was determined using spectrophotometric method (Singleton et al., 1999). The extracts in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml methanolic solution of extracts, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO3. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of NaHCO3. The samples were thereafter incubated in a thermostat at 45^{o}C for 45 min. The absorbance was determined using spectrophotometer at 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid (10-100mg/ml) and the

calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Estimation total tannins [13]

The total content of tannin in different extracts of fruits of pyrus communis was determined by folin denis method(100g of sodium sulphate+20g of phosphomalybdic acid +50 ml of phosphoric acid and 750ml of distilled water was refluxed or boiled for 2 hrs and make up the volume 1000 ml with distilled water). The colorimetric estimation of tannin is based on the measurement of blue colour formed by reduction of phosphor tungsto malybdic acid by tannin like compound in alkaline medium. 1g/ ml of extracts and standard solution of tannic acid (10-100mg/ml) was made up to 7.5 ml with distilled water. Then 0.5 ml of Folin denis reagent and 1 ml of Na_2CO_3 solution was added. The volume was made up to 10 ml with distilled water and absorbance was measured at 700 nm. The total tannic acid content was expressed mg equivalent of tannic acid per gram of extracts.

Determination of total flavonoids contents [14]

The total flavonoids content of each plant extract was estimated as per Zhishenet al[9]. In-brief, each sample (1.0ml) was mixed with 4ml of distilled water and subsequently with 0.30ml of a $NaNO_2$ solution (10%). After 5 min, 0.30 ml of an $AlCl_3$ solution (10%) was added followed by 2.0 ml of NaOH solution (1%). Immediately, after thorough mixing the absorbance was measured at 510 nm versus the blank. Standard curve of rutin was prepared(10-100mg/ml) and the results are expressed as rutin equivalents (mg ruin/gm dried extract).

Statistical analysis:

Experimental data are expressed as mean±standard error of mean (SEM). Statistical analysis was performed by two-way ANOVA followed by bonferroni posttests method of multiple comparisons was employed using Graphpad prism 5.0 software. Data were considered significant at $p < 0.001$ & $p<0.01$.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical analysis of different extracts of pyrus communis shows presence of steroid, flavonoids, glycosides, tannin, alkaloids, phenolic compound, proteins and carbohydrate. (Table 1)

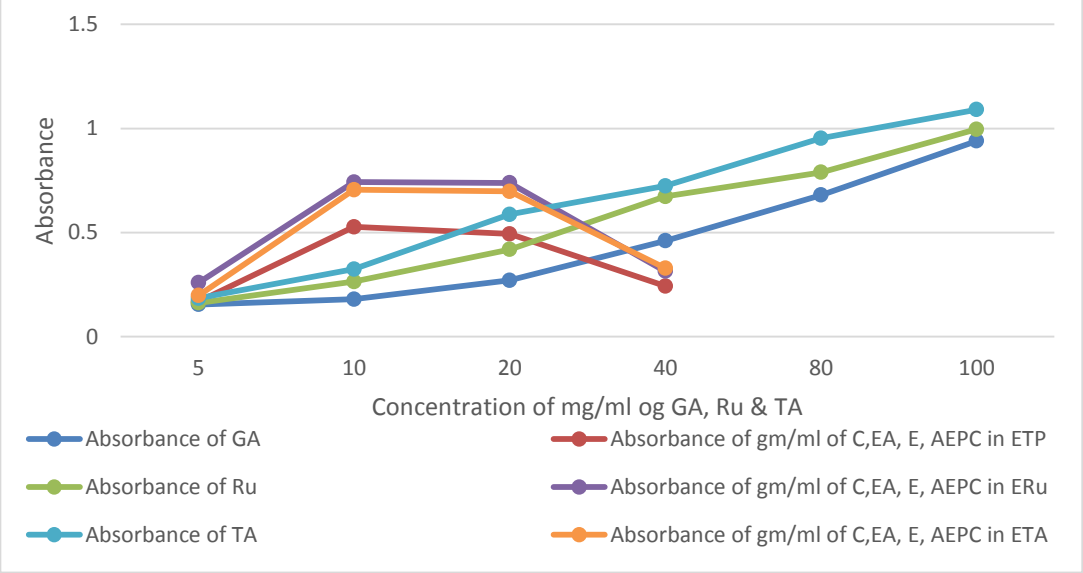
Table 1:Preliminary phytochemical screening different extract of *Pyrus communis L*.

S.No	Constituents	Tests	Chloroform	Ethyl acetate	Ethanol	Aqueous
1	Alkaloids	Mayer's test	+	+	+	+
		Dragondraff's test	+	-	-	+
		Hager's test	+	-	-	+
		Wagner's test	+	+	-	+
2	Sterols	Burchard test	+	-	-	-
		Salkowski's	+	-	-	-
3	Carbohydrates	Molisch's test	-	+	+	+
		Fehling's test	+	+	+	+
		Benedict's test	+	+	+	+
		Barfoed's test	+	-	+	+
4	Glycosodes	Legal test	-	+	+	+

		Kellerkiallani test	-	+	+	+
		Borntrager's test	-_	+	+	+
5	Fixed oils & Fats	Spot test	-	-	-	-
		Saponification test	-	-	-	-
6	Phenolic Compounds	Ferric chloride	-	+	+	+
7	Proteins & amino acids	Biuret test	+	+	+	+
		Ninhydrin test	-	+	+	-
		Millon's test	-	+	+	-
		Xanthoproteic test	-	+	+	+
		Cysteine test	-	+	-	+
		Tryptophan test	-	-	-	-
8	Terpenoids & Saponins	Foam test	-	+	+	+
		Haemolysis test	-	-	-	-
9	Tannins	Gelatin test	-	+	+	+
		$Fecl_3$ test	+	+	+	-
		Lead acetate test	-	+	+	+
10	Gums & mucilage	Mucilage test	-	+	-	+
		Hydrolytic test	-	-	-	+
11	Flavonoids	Shinoda test Conc.H_2SO_4	-	+	+	+
		lead acetate	-	+	+	+
			-	+	+	-

Where + =present, - =absent

Figure 1: The concentration response curve of GA, Ru & TA and absorbance different extracts of Pyrus communis L.



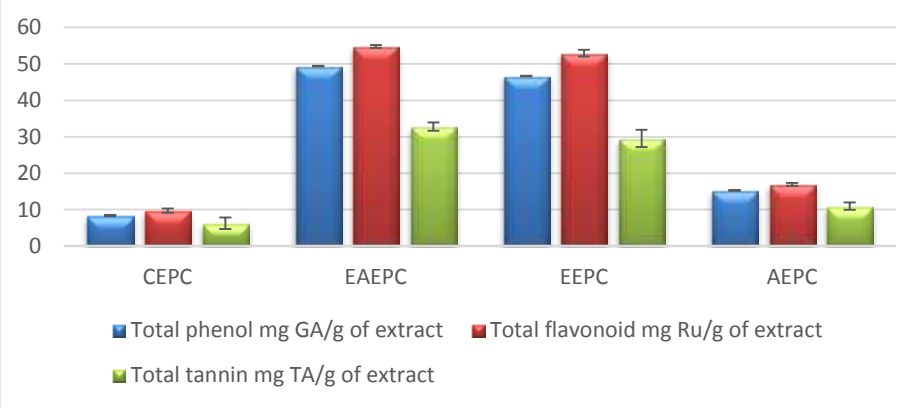
Standard curve prepared was used for the determination of total phenolic content and flavonoids using different concentrations of Gallic acid, tannic acid and rutin respectively. The total phenolic, flavonoids and tannin content in different extracts of Pyrus communis have been presented in table. Observation shows that total phenol content significantly ($p<0.001$) differ between the extracts. The content significantly was highest in the ethyl acetate extract compare to ethanol, aqueous and chloroform extract. There is no significant variation in flavonoid content between ethyl acetate and ethanol. But it is significantly differ ($p<0.001$) from aqueous and chloroform extracts. The flavonoid content in aqueous extract significantly ($P<0.001$) differ from the chloroform extract. Significantly ($p<0.001$) high concentration of tannin found in ethyl acetate and ethanol compared to aqueous and chloroform extracts. No significant variation observed in tannin content between chloroform and aqueous extracts.

Table 2: Total phenol, flavonoid and tannin content of different extracts of fruits of *Pyrus communis* L.

Extracts	Total phenol mg GA/g of extract	Total flavonoid mg Ru/g of extract	Total tannin mg TA/g of extract
Chloroform	8.38 ± 0.17^{d}	9.72 ± 0.59^{h}	6.28 ± 1.57^{j}
Ethyl acetate	49.33 ± 0.08^{a}	54.77 ± 0.41^{e}	32.76 ± 1.13^{i}
Ethanol	46.63 ± 0.12^{b}	52.92 ± 0.94^{e}	29.52 ± 2.34^{i}
Aqueous	15.27 ± 0.03^{c}	16.92 ± 0.38^{g}	10.96 ± 1.02^{j}

Values are expressed as mean± SEM of three replicates. Mean bearing same superscripted do not differ significantly. Mean bearing different superscripted differ significantly. The different superscripted 'a'values have significantly differ ($p<0.001$) from' b, c'& d. 'b' indicates significantly differ ($p<0.001$) from 'c & d'. 'c' indicates significantly differ ($p<0.001$) from 'c'. 'e' differ from ($p<0.001$) 'g & h'. 'g' differ from ($p<0.001$)'h'. 'i' significantly differ from ($p<0.001$) 'j'

Figure 2: Total phenol, flavonoid and tannin content of different extracts of fruits of *Pyrus communis L*.



Values are expressed as mean± SEM of three replicates. Mean bearing same superscripted do not differ significantly. Mean bearing different superscripted differ significantly. The different superscripted 'a'values have significantly differ ($p<0.001$) from' b, c'& d. 'b' indicates significantly differ ($p<0.001$) from 'c & d'. 'c' indicates significantly differ ($p<0.001$) from 'c'. 'e' differ from ($p<0.001$) 'g & h'. 'g' differ from ($p<0.001$)'h'. 'i' significantly differ from ($p<0.001$) 'j'

DISCUSSION

These medicinal plants are rich sources for naturally occurring antioxidants especially phenolic and flavonoids contents. These agents have ability to scavenge free radicals, super oxide and hydroxyl radicals, etc thus they enhance immunity and antioxidant defense of the body [15]. Dietary supplementation of these compounds reduces the oxidative damage to cell membrane lipid, protein and nucleic acid due strong quenching property of free radicals [16].

For acceptance of medicinal plants into scientific medicine, it is necessary that their effectiveness and safety be evaluated and confirmed through active ingredient testing. To maximize the extractive capability of phenolic and flavonoids components from plant material is considerably depended on the type of solvent. Highest content of phenolic, flavonoids and tannin in ethanolic and ethyl acetate extract in comparison to other solvents used, make this organic solvent (ethanol) an ideal and selective to extract a great number of bioactive phenolic compounds. Similarly, Mohammedi [17]. Collagen fibers treated with the plant flavonoid, catechin, have been found to be stable. Such stabilization effect has been shown to involve hydrogen bonding and hydrophobic interactions [18].

Tannins are generally defined as naturally occurring polyphenolic compounds of high molecular weight to form complexes with the proteins. Tannins are important source of protein in animals but unfortunately the amounts of tannins that they contain vary widely and largely unpredictably, and their effects on animals range from beneficial to toxicity and death [19]. The toxic or anti-nutritional effects tend to occur in times of stress when a very large proportion of the diet having high concentration of tannins. Thus consumption of foods naturally having antioxidant activity is the most efficient way of combating such tissue injuries, undesired transformations and preventing health risks [20]. Tannins are phenolic compounds that typically act as astringents and are found in a variety of herbal products used for wound healing. This astringent property is responsible for wound contraction and increased rate of epithelialization at the granulation formation and scar remolding phases [21]. Accordingly, topical treatment with a tannin rich fraction of the bark of *Terminalia arjuna* was found to demonstrate significant increase in the tensile strength of the incision wounds. The maximum tensile strength was developed by tannins-fraction treated rats (719 g, compared to the standard reference formulation Betadine (609g) [22].

CONCLUSION

In present study the ethyl acetate and ethanolic extract have high concentration of flavonoids, tannin and phenolic concentration. Therefore, ethyl acetate and ethanolic extract of *Pyrus communis* have greater potential to produces more beneficial effects or pharmacological important as compared to other extracts.

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