

Nutraceutical studies on *Eriobotrya japonica* (Thunb.) Lindl. (Fruits & Seeds)

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Abstract

Fruits and vegetables have plenty of natural antioxidants, especially vitamin C and E. Fruits contained beta-carotene, phenolic compounds such as anthocyanin and other flavonoids, which showcase a wide range of biological benefits, including antioxidant, anti-inflammatory and anti carcinogenic properties. *Eriobotrya japonica* commonly called as loquat fruit is a subtropical evergreen fruit. In the present study an attempt was made to analyzed the phytochemical screening, nutraceuticals and antioxidant activity of the aforesaid traditionally important and pharmacologically potent fruits and seeds of *E. japonica*. The dried powdered fruits and seed samples were subjected for the analysis of nutritional parameters, phytochemical screening and evaluation of antioxidant activity. The nutritional parameters such as total carbohydrate, total starch, total proteins and levels of free amino acids were analyzed. Phytochemical screening of *E. japonica* clearly showed the presence of certain important secondary metabolites such as alkaloids, phenols, flavonoids, glycosides, cardiac glycosides, phytosterol and gums and mucilage. The antioxidant activity of the plant extracts were determined by using super oxide free radical scavenging assay. The results of the present study clearly indicated that methanol seed extract showed better free radical scavenging activity at concentration of 1000 mg/ml of extract. Among the selected samples fruits of *E. japonica* were found to be more potent than the seeds of the same species. However, more advanced pharmacological and clinical studies would be required to investigate *in vivo* mechanism of pharmacological effects of this important edible fruits *E. japonica*.

Keywords: Nutraceuticals, Phytocompounds, Antioxidant activity and *E. japonica*.

1. Introduction

Rosaceae - rose" family, is a medium sized family of flowering plants, including 4,828 known species in 91 genera. They are very well represented in China, with great economic and scientific importance. Loquat (*Eriobotrya japonica* (Thunb) Lindl) a subtropical evergreen fruit tree of the Rosaceae family Maloideae, family originated in southwest China and has been cultivated for over 2,000 years [1]. It has been introduced to more than 30 countries including Japan, Mediterranean countries in Europe, India, Australia, New Zealand, Madagascar, and South Africa, while commercial cultivation is limited to a few countries. In China, loquat blooms in the fall and early winter, and the fruit ripens between May and June. Loquat leaves and fruits are traditionally used for treating coughs and as

expectorant, and the flower is an excellent source of honey. Many plants of this family are of economic importance and contribute to peoples livelihoods [2]. The Rosaceae contain a great number of fruit trees of temperate regions. The fruits contain vitamins, acids, and sugars and can be used both raw and for making preserves, jam, jelly, candy, various drinks, wine, vinegar, etc. The dried fruits of the genera *Amygdalus* and *Armeniaca* are of high commercial value. Some plants in the genus *Rosa* containing essential oils or with a high vitamin content are used in industry. Stems and roots are used for making tannin extract, and young leaves are used as a substitute for tea. The Rosaceae include many well known and beloved species of economic importance particularly edible temperate zone fruits [3] and ornamentals, but also some timber crops and medicinals or

nutraceuticals. The aim of this study is to investigate the nutritional parameters, phytochemical screening and antioxidant activity of the fruit and seed of *E. japonica*.

1.1 Objectives of the Study

- 1) To collect the fresh fruits and seeds of *E. japonica* from natural strands of The Nilgiris, Tamil Nadu.
- 2) To extract the phytochemical compounds present in fruits and seeds by using petroleum ether, chloroform, methanol, water as solvent by maceration method.
- 3) To evaluate the nutritional composition of fruit and seed.
- 4) To find out the different phytochemical constituents of selected fruit and seeds through phytochemical screening.
- 5) To quantify the total phenolic and flavonoid content of the selected fruit and seeds
- 6) To evaluate the antioxidant activity of crude extracts using superoxide radical scavenging assay.

2. Materials and methods

2.1. Collection and identification of plant material

The fresh plant materials were collected during the month of June 2016 in the natural strands of Gandhinagar, Gudalur, The Nilgiris district, Tamil Nadu, India (Plate 1).

The taxonomic identity of the plant was confirmed by Botanical Survey of India, TNAU campus, Southern Circle, Coimbatore, Tamil Nadu. The authentication number is (BSI/SRC/5/23/2016/TECH/897).

Plate-1: *Eriobotrya japonica* (Thunb) Lindl



The plant materials were washed under running tap water to remove the surface pollutants. The selected plant parts were air dried under shade condition and samples were powdered and used for further studies.

2.2. Crude extract preparation

2.2.1. Solvent extraction

Fifty grams of dried powder of the samples were mixed with 200 ml of each solvent (petroleum ether, chloroform, methanol and water). The mixture was stirred in a shaker (LABTRON Ls-100) at 3000 rpm at room temperature for 38 hrs. The extracts were filtered and solvents were evaporated using a rotary evaporator at 50°C. The extracts were stored at -20°C until testing [4].

2.2.2. Extract recovery percentage

The amount of crude extract recovered after successive extraction was weighed and the percentage of yield was calculated by the following formula,

$$\text{Extract recovery percent} = \frac{\text{Amount of extract recovered (g)}}{\text{Amount of plant sample (g)}} \times 100$$

2.3. Nutritional parameters

2.3.1. Extraction and Estimation of total Carbohydrate and total Starch

The carbohydrate was estimated by the method of Sadasivam and Manickam, [5] using glucose as a standard in anthrone reagent method.

2.3.2. Extraction and Estimation of Proteins (True Proteins)

The protein was estimated as described by Lowry *et al.*, [6] using Bovine serum albumin as a standard.

2.3.3. Determination of total free amino acids

Free amino acids were estimated by Ninhydrin method [7].

2.4 Preliminary phytochemical screening

Qualitative phytochemical screening of petroleum ether, chloroform, methanol and water extracts of *E. japonica* of fruit and seeds were carried out by standard methods [8].

2.5. Quantitative estimation of phytocompounds

2.5.1. Estimation of total phenols

The total phenolics of the different plant extracts were determined according to the method described by Makkar [9]. In this method 150 µL of different plant extracts were taken into a series of test tubes and made up to 1 mL with distilled water. A test tube with 1 mL of distilled water served as the blank. Then, 500 µL of Folin – Ciocalteu Phenol reagent (1 N) was added to all the test tubes including the blank. After 5 minutes, 2.5 mL of sodium carbonate solution (20%) was added to all the test tubes. The test tubes were vortexed well to mix the contents and incubated in dark for 30 minutes. The formation of blue colour in the incubated test tubes indicated the presence of phenolics. Soon after incubation the absorbance was read at 725 nm against the reagent blank. Gallic acid standard was also prepared and the results were expressed as Gallic acid equivalents (GAE). The analyses were performed in triplicates.

2.5.2. Estimation of total flavonoids

The flavonoid contents of all the extracts were quantified according to the method described by Zhishen *et al* [10]. About 800 µL of all the plant extracts were taken in different test tubes and 2 mL of distilled water was added to each test tube. A test tube containing 2.5 mL of distilled water served as blank. Then, 150 µL of 5% NaNO₂ was added to all the test tubes followed by incubation at room temperature for 6 minutes. After incubation, 150 µL of 10%

AlCl₃ was added to all the test tubes including the blank. All the test tubes were incubated for 6 minutes at room temperature. Then 2 mL of 3% NaOH was added to all the test tubes which were then made up to 5 mL using distilled water. The contents in all the test tubes were vortexed well and they were allowed to stand for 15 minutes at room temperature. The pink colour developed due to the presence of flavonoids was read spectrophotometrically at 510 nm. Rutin was used as the standard for the quantification of flavonoids. All the experiments were done in triplicates and the results were expressed in Quercetin equivalents (QE).

2.6. Antioxidant Assays

The antioxidant activity of crude extracts was determined by standard *in-vitro* method. 1. Superoxide radical scavenging assay.

2.6.1. Superoxide radical scavenging activity

The assay was based on the capacity of various extracts to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin–light–NBT system (Beauchamp and Fridovich [11]). About 3 mL of reaction mixture containing 50 mM sodium phosphate buffer (pH-7.6), 20 µg riboflavin, 12 mM EDTA and 0.1 mg NBT was added to 100 µL sample solution, BHT and rutin. Reaction was started by illuminating the reaction mixture with samples for 90 seconds. The illuminated reaction mixture without sample was used as the negative control. Immediately after illumination, the absorbance was measured at 590 nm against the blank (unilluminated

reaction mixture without plant sample). The scavenging activity on superoxide anion generation was calculated as: Scavenging activity (%) =

$$[(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] \times 100.$$

3. Results and discussion

3.1 Nutritional Analysis

The data on the nutritional parameters of the selected fruit and seed samples were investigated and the results were presented in Table- 1.

3.1.1 Total carbohydrates

The total carbohydrate content of the presently investigated fruit and seed samples were found to be 0.3215 g/100g and 0.3003 g/100g respectively.

3.1.2 Total Starch

The data on total starch content of dried fruit and seed powder materials of *E. japonica* exhibited the presence of considerable levels of total starch (0.010842 and 3.012153 g/100g of dried fruit and seed powder).

3.1.3 Total (true) protein

The data on total true protein of dried fruit and seed powder materials of *E.japonica* contained substantial levels of total protein 1.5065 g/100g of fruit powder and 0.0753 g/100g of seed powder. The fruit has registered the highest level of total protein.

3.1.4 Total amino acid

The amino acid content of the presently investigated samples were found to be 0.0039 g/100g and 0.0352 g/100g of dried fruit and seed samples of *E. japonica* respectively.

Table 1: Data on nutritional parameters of fruit and seed sample of *E.japonica* (g/ 100g)*

S. No	Nutrition	Plant extract	
		fruit	Seed
1	Carbohydrate	0.321517 ± 0.007063	0.300373 ± 0.009872
2	Starch	3.012153 ± 0.010842	1.137153 ± 0.021049
3	Protein	1.506591 ± 0.090807	0.07533 ± 0.133498
4	Amino acid	0.003903 ± 0.025166	0.035237 ± 0.001528

* All values are mean of triplicate determinations expressed on dry weight basis.

In the present study *E.japonica* fruit sample exhibited higher level of protein content comparable with the earlier reports on *Bauhinia purpurea* (Fabaceae) *Diplazium esculentum* Retz. Sw. (Athyriaceae) *Elaeagnus latifolia* (Elaeagnaceae) *Elaeagnus pyriformis* [12]; *Persea americana* fruit [13]; *R. ellipticus* [14].

3.2 Phytochemical screening

3.2.1. Qualitative analysis of phytochemicals

The selected plant powder samples were subjected to qualitative organic analysis table-2. Phytochemicals of Carbohydrate, protein, amino acid, alkaloids, phenols, flavonoids, glycosides, cardiac glycosides, phytosterol and gum and mucilage was positively answered in fruit and seed samples of *E. japonica*.

Table 2: Preliminary phytochemical screening of fruit and seed powder of *E.japonica*

S. No.	Phytoconstituents	<i>E.japonica</i>	
		Fruit	Seed
1	Alkaloid	+	+
2	Carbohydrate	+	+
3	Protein	+	+
4	Amino Acid	+	+
5	Phenol	+	+
6	Saponin	+	+
7	Glycosides	+	+
8	Flavones glycosides	+	+
9	Gum and mucilage	+	+
10	Cardiac glycosides	+	+
11	Fixed oil and fats	+	+

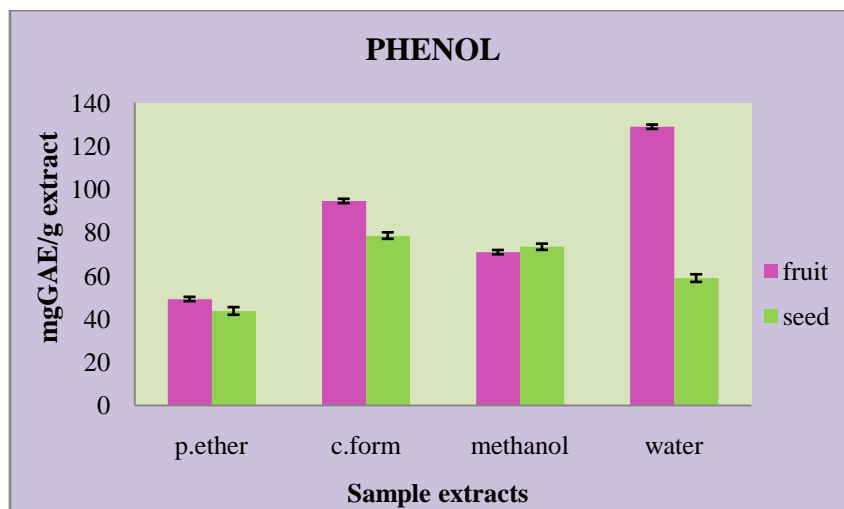
(+) Indicates positive (--) Indicates negative

3.2.2. Quantitative estimation of phytoconstituents (total phenols)

Selected samples using various solvent extracts were prepared to examine the major phytoconstituents. In the

present study total phenols content of fruits and seeds were quantified. Among the investigated samples fruits were exhibited highest (128.8333 mg/g) levels of phenol content then the seed sample Fig-1.

Figure 1: Total Phenol content of fruits and seeds sample of *E. Japonica*

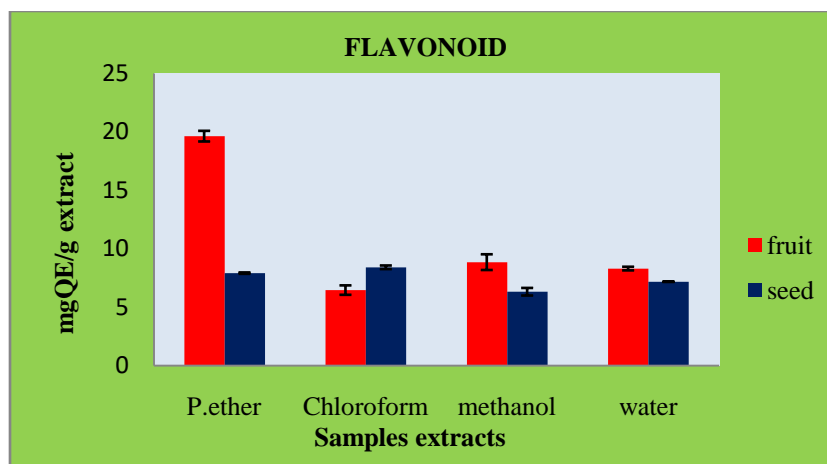


3.2.3 Quantitative estimation of flavonoids

Flavonoid content of the fruits and seeds sample of *E. japonica* were quantified. Among the investigated

samples, In fruit sample the flavonoid content was registered in very high level when compare to the seed sample result showed in fig-2.

Figure 2: Total flavonoid content of fruits and seeds samples of *E. Japonica*

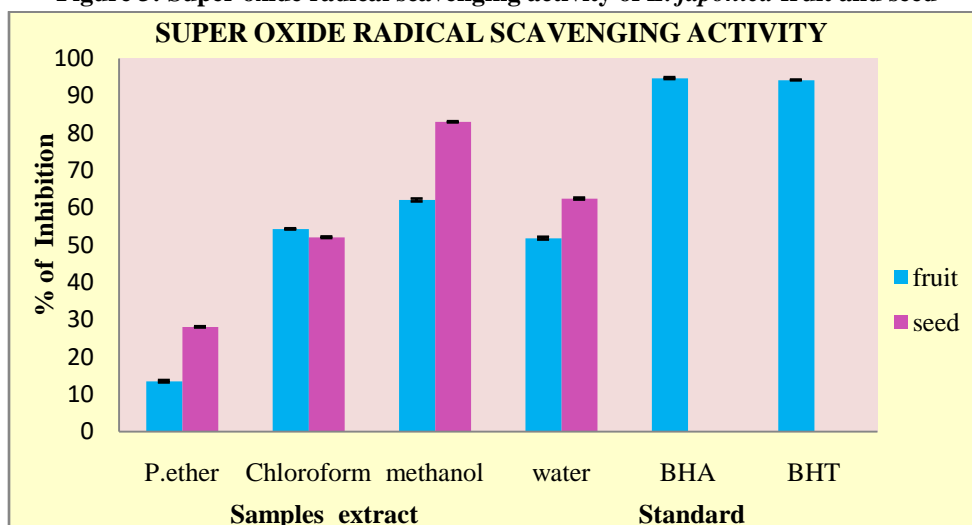


3.3. Antioxidant activity

3.3.1. Superoxide radical scavenging activity

The superoxide free radical scavenging assay of selected plant sample was given in figure-3, and it was found to be ranged from 13.50 to 82.99%. Among the selected

samples, the methanol extract of seed sample (82.99%) has exhibited the highest rate of free radical scavenging activity. This result was almost similar to that of Butylated Hydroxy Anisole and Butylated Hydroxy Toluene.

Figure 3: Super oxide radical scavenging activity of *E. japonica* fruit and seed

The antioxidant ability and radical scavenging properties of plants are associated with its medicinal values. In antioxidant assays, superoxide radical scavenging activity was found to be more effective free radical inhibition activity. Methanol extract of seed sample of *E. japonica* found to exhibit more effective free radical inhibition activity against superoxide radical scavenging activity. The free radical inhibition activity of crude methanolic extract of selected powder samples was ranged between 13.50 and 82.99% which is in agreement with that of the previous reports in *Rubus ellipticus* [15]; (*Chrysophyllum albidum*), Hog plum (*Spondias mombin.*), Bush mango (*Irvingia gabonensis*) and Monkey cola (*Cola millenii*) [16].

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