ORIGINAL ARTICLE

Physicochemical analysis of leaves of *Eriobotrya japonica* and antioxidant and antidiabetic evaluation of its methanolic extract

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Abstract

Background: *Eriobotrya japonica* is a traditional medicinal plant known as loquat and used in East Asian countries such as Japan, China, Korea, India, Nepal, and Pakistan. However, standards for *E. japonica* are not published in any of the Indian monographs as the quality of plant-based products may be affected by factors such as location, climate, cultivation, and collection. **Objective:** The objective of the study is to develop the analytical profile and *in vitro* evaluation of antioxidant and antidiabetic potential. **Materials and Methods:** *E. japonica* was subjected to organoleptic, physicochemical, qualitative, and chromatographic evaluation. Further, antioxidant and antidiabetic activities were also evaluated using 2,2-diphenylpicrylhydrazyl and α-amylase inhibition activity, respectively. **Results and Discussion:** The average results of the loss on drying, total ash, acid-insoluble ash, water-soluble extractive value, and alcohol-soluble extractive values were 7.08 ± 0.58%, 8 ± 0.54%, 1 ± 0.31%, 21.6 ± 1.89%, and 11.06 ± 1.28%, respectively. Only one principle spot (Rf = 0.5) was recorded in the thin layer chromatography of *E. japonica.* The observed inhibitory concentration 50% (IC_{50}) of the extract was 0.453 mg/mL whereas IC_{50} of ascorbic acid (standard) was 0.528 mg/mL. For antioxidant activity, a similar trend was observed in the IC_{50} of extract 0.015 mg/mL to IC_{50} of acarbose (standard) 0.058 mg/ml for α-amylase inhibition activity. **Conclusion:** The results suggest that *E. japonica* can have good potential in the treatment of diabetes.

Key words: Amylase, antidiabetic activity, antioxidant activity, *Eriobotrya japonica*

INTRODUCTION

Eriobotrya japonica is a traditional medicinal plant known as loquat, and its different parts are used to treat diverse pathophysiology and also consumed as a food product in daily life. Generally, it is used in East Asian countries such as Japan, China, Korea, India, Nepal, and Pakistan. In India, it is mainly used in Uttar Pradesh, Punjab, and Himachal Pradesh. The traditional healers and Vaidyas of Punjab are using the juice of leaves of *E. japonica* to treat diabetes. Moreover, recent studies based on the antidiabetic effect of *E. japonica* have been suggesting its role to reduce blood glucose.[1-3] It also reported to have antioxidant, antiviral,[2] neuroprotection,[4,5] cardiovascular health,[6] glucose metabolism,[3,7-9] antiobesity,[10] bone and joint strength,[11] anti-inflammatory,[12-15] hormonal activity,[16,17] peripheral organ systems,[18-22] and cancer metabolism activities.[23-25]

E. japonica is a small, short-trunked, upward-branching, broadleaf evergreen tree that typically grows to 10–25' tall

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in a round form [Figure 1]. It also grows as a huge shrub. Generally, it is known for its compact size, foliage, flowers, and fruits.

Leaves are lanceolate, oblong to wide, length 12–30 cm, width 4–9, and pointed apex, and the leaf base is wedge-shaped, serrate margin with short petiole; color of upper surface is green to green-brown whereas lower surface is light green-brown showing the presence of brown woolly hairs; vein is prominent at lower surface and color is light yellow-brown [Figure 2]; it is slight odor and tasteless. The fragrance of the flower is sweet, petals are five in number, the color of the flower is white, panicles are large (to 6" long), and it blooms between October and November.

Fruits are small, spherical (pear-shaped); the length is 1–2" long, juicy, and fleshy. Fruits ripen in the spring season. Color of the fruit is yellow to orange skin, whereas seeds are one to several in number and large.[26]

The monograph of *E. japonica* is mentioned in the Japanese Pharmacopoeia. However, standards for *E. japonica* is not published in any on the Indian monographs as the quality of plant-based products may be affected by factors such as location, climate, cultivation, and collection. Hence, there is a need to develop the standards for *E. japonica* as per the climatic conditions on India. Hence, the present study was designed to develop the physicochemical, qualitative, and chromatographic standards for *E. japonica*. Further, the extract was also evaluated for the *in vitro* antioxidant and antidiabetic activity.

Figure 1: Habitat of *Eriobotrya japonica* is an evergreen plant

Figure 2: Leaves of *Eriobotrya japonica*

MATERIALS AND METHODS

Plant Material

The leaves of *E. japonica* were collected from the village Kotla Naudh Singh District. Hoshiarpur (Punjab), and authenticated from National Institute of Pharmaceutical Education and Research, Mohali.

Organoleptic Study

The leaves of *E. japonica* were observed for their organoleptic parameters, including color, odor, taste, and texture.

Physicochemical Analysis

Various physicochemical parameters were analyzed to find out the identity, purity, and strength of the *E. japonica*, i.e., foreign matter, loss on drying, total ash, acid-insoluble ash, water-soluble extractive value, and alcohol-soluble extractive value.[27,28] All tests were repeated for six times.

Qualitative Analysis

Various chemical constituents were analyzed in aqueous and alcoholic extract for the phytochemical screening to establish a chemical profile of *E. japonica*, including flavonoids, alkaloids, tannins, phenol, reducing sugars, saponins, protein, phytosterols, and glycosides.[29]

Extraction of the *E. japonica*

The fresh leaves of *E. japonica* were crushed and extracted with methanol using the Soxhlet extraction method for 24 h. The obtained extract was filtered, and the filtrate was evaporated on a rotary evaporator.[30]

Thin Layer Chromatography

Thin layer chromatography (TLC) was used for the qualitative analysis by enhancing the separation and resolution of the compounds with a fine particle size of stationary phase.[31] The mobile phase was used as a mixture of water:acetonitrile (3:2), and dilute sulfuric acid (10%) was used as a spray reagent.[32]

Preparation of Sample

Ten milligrams of dry methanolic extract of *E. japonic*a was taken and dissolved in 10 ml of the methanol. Thereafter, it was filtered by using Whatman filter paper and the filtrate was concentrated on a water bath and stored in a closed container.

In vitro Antioxidant Activity

2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging assay

200 µl of analytical sample was added into a test tube containing 800 µl of 0.1 M Tris-HCl buffer (pH 7.4). In this solution, 1 ml of ethanolic DPPH solution was added immediately and test tube was shaken for 10 seconds. Then solution kept in dark place at room temperature for 30 minutes, and thereafter absorbance was recorded at 517 nm. For blank 1.2 ml of ethanol and 800 µl 0.1M Tris-HCl buffer were taken and absorbance was recorded at 517 nm. All samples were done in triplicates. Percentage of inhibition was calculated by using following formula.

$$\%\ \text{Inhibition}\ (\%\ \text{I}) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}} \times 100$$

In Vitro α-Amylase Inhibition Assays (Antidiabetic Activity)

Starch-iodine method was used for the determination of α-amylase inhibition activity. A 10 µL of α-amylase solution (0.025 mg/ml) was mixed with 390 µL of phosphate buffer containing different concentrations of methanolic extract of *E. japonica*. After incubation at 37°C for 10 min, 100 µL of the 1% starch solution was added and re-incubated for 1 h. After re-incubation, 0.1 mL of 1%, iodine solution was added, and further, it was diluted with 5 ml distilled water. The absorbance of all the solutions was measured at 565 nm,[33] and % inhibition was calculated by the following formula.

$$\%\ \text{Inhibition}\ (\%\ \text{I}) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}} \times 100$$

RESULTS AND DISCUSSION

Organoleptic Characteristics

The organoleptic characteristics of raw material, such as color, odor, taste, and texture are mentioned in Table 1. The plant leaves have dark green color at the dorsal surface whereas light green at the ventral surface with a smooth texture. Odor found to be a characteristic, and the taste was bitter.

Physicochemical Study

Standards of the physicochemical analysis of *E. japonica* were not found in Ayurvedic Pharmacopoeia of India. However, physicochemical parameters were performed during the study, and the results of the analysis are mentioned in Table 2. No foreign matter was found in the sample. The average results of the loss on drying, total ash, acid-insoluble ash, water-soluble extractive value, and alcohol-soluble extractive value were 7.08 ± 0.58%, 8 ± 0.54%, 1 ± 0.31%, 21.6 ± 1.89%, and 11.06 ± 1.28%, respectively.

Phytochemical Screening for *E. japonica*

Qualitative analysis of *E. japonica* revealed the presence of tannins, flavonoids, coumarins, steroidal glycosides, alkaloids, protein, quinones, saponins, and reducing sugars. The results of qualitative tests are mentioned in Table 3. A methanolic extract found to contain a similar composition of phytochemical. Saponins were absent in the extracts.

Chromatographic Analysis

One principle spot Rf was recorded in the TLC of *E. japonica* extract in visible daylight after spray. However, under the short UV wavelength and long UV wavelength, there was no sport observed, as represented in Table 4, and Figure 3.

Table 1: Observation of organoleptic study of *Eriobotrya japonica* leaves

Parameters	Observation
Color	Dark green (dorsal surface) Light green (ventral surface)
Odor	Characteristic
Taste	Slight bitter
Texture	Smooth

Figure 3: Image of thin layer chromatography analysis of *Eriobotrya japonica* leaves methanolic extract in water:acetonitrile (3:2)

Table 2: Observation during the pharmaceutical analysis of *Eriobotrya japonica* leaves

Parameters	Batches (%)						SD
	I	II	III	IV	V	VI	
Foreign matter	0	0	0	0	0	0	0
Loss on drying	7.5	7	7.5	7.5	6	7	7.08±0.58
Total ash	7.5	9	8	8	7.5	8	8±0.54
Acid-insoluble ash	1	1.5	1	0.5	1	1	1±0.31
Water-soluble extractive	22.4	18.4	20.8	21.4	24	22.4	21.6±1.89
Alcohol-soluble extractive	12	9.6	9.6	11.2	11.2	10.4	11.06±1.28

SD: Standard deviation

Table 3: Observation of physicochemical study of *Eriobotrya japonica* leaves

Components	Chemical tests	Observation	Methanolic extract
Flavonoids	Lead acetate tests	Yellow color	+ve
	Shinoda test	Orange color	–ve
Alkaloids	Mayer's test	Red precipitates	+ve
	Dragendroff's test	Red precipitates	–ve
	Wagner test	Brow reddish precipitates	+ve
Tannins	Ferric chloride test	Greenish black color	+ve
Phenol	Ferric chloride test	Greenish black color	–ve
Reducing sugars	Fehling's test	Brick red color	+ve
Saponins	Foam test	Foam absent	–ve
Protein	Xanthoprotein test	Yellow color	+ve
Phytosterols	Salkowski test	Golden yellow color	–ve
	Lieberman Burchardt test	Brown green, red junction	+ve
Glycosides	Kellar Killani's test	Brown ring at the junction	+ve

Table 4: Thin layer chromatography analysis *Eriobotrya japonica* leaves methanolic extract

Sample	Solvent system	Rf value			Required principle spot red-purple color
		Short UV	Long UV	After spray evenly dilute sulfuric acid	
Eriobotrya japonica	Water: acetonitrile (3:2)	Not visible	Not visible	0.5	Purple in color

UV: Ultraviolet

Table 5: Percentage inhibition of ascorbic acid, *Eriobotrya japonica* methanolic extract

Concentration (mg/ml)	Percentage inhibition of standard	Percentage inhibition of extract
0.075	14.87	18.67
0.15	32.27	35.75
0.3	41.77	43.67
0.45	47.15	51.26
0.6	50.94	52.84
0.75	55.37	57.27

In Vitro 2,2-diphenylpicrylhydrazyl Assay

Different concentrations of ascorbic acid as standard and *E. japonica* methanolic extracts were used to check its antioxidant activity by DPPH assay. Inhibitory concentration 50% (IC_{50}) of extract was 0.453 mg/mL whereas IC_{50} of ascorbic acid (standard) was 0.528 mg/mL. % inhibition of extract was comparatively high than standard [Table 5 and Figure 4].

Table 6: α-amylase inhibition of acarbose and *Eriobotrya japonica* methanolic extract

Concentration (mg/ml)	Percentage inhibition (acarbose)	Percentage inhibition (*Eriobotrya japonica* methanolic extract)
0.075	58.5	73.13
0.15	67.76	78.05
0.3	74.92	91.34
0.45	86.86	100.58
0.6	100.34	100.89
0.75	100.74	100.44

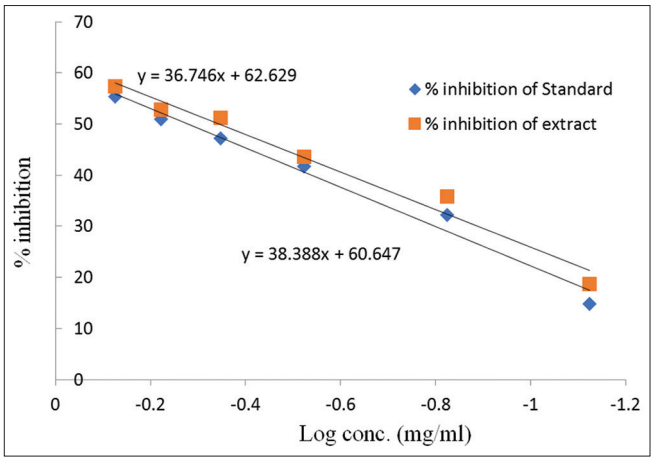

Figure 4: % inhibition ascorbic acid, *Eriobotrya japonica* extract, and its self nanoemulsifying drug delivery system

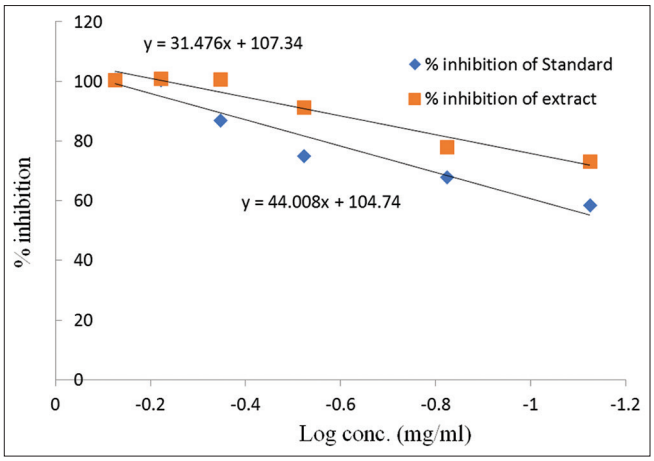

Figure 5: α-amylase inhibition of acarbose and *Eriobotrya japonica* methanolic extract

α-Amylase Inhibition Assay

To evaluate and compare the anti-diabetic activity, α-amylase assay was performed. The extract had high % inhibition as compared to acarbose. IC_{50} of extract was 0.015 mg/mL whereas IC_{50} of acarbose (standard) was 0.058 mg/ml [Table 6 and Figure 5].

CONCLUSION

E. japonica is a traditional medicinal plant, and its different parts are used to treat diverse pathophysiology and also consumed as a food product in daily life. The present study was focused on the development of its physicochemical, qualitative, and chromatographic standards, including the antioxidant and antidiabetic effect profile. The average results of the loss on drying, total ash, acid-insoluble ash, water-soluble extractive value, and alcohol-soluble extractive vale were 7.08 ± 0.58%, 8 ± 0.54%, 1 ± 0.31%, 21.6 ± 1.89%, and 11.06 ± 1.28%, respectively. Only one principle spot (Rf = 0.5) was recorded in the TLC of *E. japonica* methanolic extract in visible daylight after spray. Moreover, the antioxidant activity and antidiabetic effect of *E. japonica* were comparable with standards in DPPH inhibition and α-amylase inhibition, respectively. The observed IC_{50} of the extract was 0.453 mg/mL, whereas the IC_{50} of ascorbic acid (standard) was 0.528 mg/ml. For antioxidant activity, a similar trend was observed in the IC_{50} of extract 0.015 mg/mL to IC_{50} of acarbose (standard) 0.058 mg/ml for α-amylase inhibition activity. The results suggest that *E. japonica* can have good potential in the treatment of diabetes.

REFERENCES

1. Noreen W, Wadood A, Hidayat HK, Wahid SA. Effect of *Eriobotrya japonica* on blood glucose levels of normal and alloxan-diabetic rabbits. Planta Med 1988;54:196-9.
2. Bhogireddy N, Naga A, Ramesh B, Pradeep M. Anti-inflammatory and anti-diabetic activities with their other ethnomedicinal properties of the plants. J Med Plants Stud 2013;1:87-96.
3. Shih CC, Ciou JL, Lin CH, Wu JB, Ho HY. Cell suspension culture of *Eriobotrya japonica* regulates the diabetic and hyperlipidemic signs of high-fat-fed mice. Molecules 2013;18:2726-53.
4. Kim MJ, Lee J, Seong AR, Lee YH, Kim YJ, Baek HY, *et al.* Neuroprotective effects of *Eriobotrya japonica* against β-amyloid-induced oxidative stress and memory impairment. Food Chem Toxicol 2011;49:780-4.
5. Cha DS, Eun JS, Jeon H. Anti-inflammatory and antinociceptive properties of the leaves of *Eriobotrya japonica*. J Ethnopharmacol 2011;134:305-12.

6. Tanaka K, Tamaru S, Nishizono S, Miyata Y, Tamaya K, Matsui T, *et al.* Hypotriacylglycerolemic and antiobesity properties of a new fermented tea product obtained by tea-rolling processing of third-crop green tea (*Camellia sinensis*) leaves and loquat (*Eriobotrya japonica*) leaves. Biosci Biotechnol Biochem 2010;74:1606-12.
7. Kotowaroo MI, Mahomoodally MF, Gurib-Fakim A, Subratty AH. Screening of traditional antidiabetic medicinal plants of Mauritius for possible alpha-amylase inhibitory effects *in vitro*. Phytother Res 2006;20:228-31.
8. Qa'dan F, Verspohl EJ, Nahrstedt A, Petereit F, Matalka KZ. Cinchonain Ib isolated from *Eriobotrya japonica* induces insulin secretion *in vitro* and *in vivo*. J Ethnopharmacol 2009;124:224-7.
9. Lü H, Chen J, Li WL, Ren BR, Wu JL, Zhang HQ, *et al.* Hypoglycemic effect of the total flavonoid fraction from folium *Eriobotryae*. Phytomedicine 2009;16:967-71.
10. Zong W, Zhao G. Corosolic acid isolation from the leaves of *Eriobotrta japonica* showing the effects on carbohydrate metabolism and differentiation of 3T3-L1 adipocytes. Asia Pac J Clin Nutr 2007;16 Suppl 1:346-52.
11. Choi YG, Seok YH, Yeo S, Jeong MY, Lim S. Protective changes of inflammation-related gene expression by the leaves of *Eriobotrya japonica* in the LPS-stimulated human gingival fibroblast: Microarray analysis. J Ethnopharmacol 2011;135:636-45.
12. Alshaker HA, Qinna NA, Qadan F, Bustami M, Matalka KZ. *Eriobotrya japonica* hydrophilic extract modulates cytokines in normal tissues, in the tumor of meth-A-fibrosarcoma bearing mice, and enhances their survival time. BMC Complement Altern Med 2011;11:9.
13. Uto T, Suangkaew N, Morinaga O, Kariyazono H, Oiso S, Shoyama Y, *et al.* Eriobotryae folium extract suppresses LPS-induced iNOS and COX-2 expression by inhibition of NF-kappaB and MAPK activation in murine macrophages. Am J Chin Med 2010;38:985-94.
14. Kim SH, Kwon YE, Park WH, Jeon H, Shin TY. Effect of leaves of *Eriobotrya japonica* on anaphylactic allergic reaction and production of tumor necrosis factor-alpha. Immunopharmacol Immunotoxicol 2009;31:314-9.
15. Yokota J, Takuma D, Hamada A, Onogawa M, Yoshioka S, Kusunose M, *et al.* Gastroprotective activity of *Eriobotrya japonica* seed extract on experimentally induced gastric lesions in rats. J Nat Med 2008;62:96-100.
16. Kang SC, Lee CM, Choi H, Lee JH, Oh JS, Kwak JH, *et al.* Evaluation of oriental medicinal herbs for estrogenic and antiproliferative activities. Phytother Res 2006;20:1017-9.
17. Gumy C, Thurnbichler C, Aubry EM, Balazs Z, Pfisterer P, Baumgartner L, *et al.* Inhibition of 11beta-hydroxysteroid dehydrogenase type 1 by plant extracts used as traditional antidiabetic medicines. Fitoterapia 2009;80:200-5.
18. Yokota J, Takuma D, Hamada A, Onogawa M, Yoshioka S, Kusunose M, *et al.* Scavenging of reactive oxygen species by *Eriobotrya japonica* seed extract. Biol Pharm Bull 2006;29:467-71.
19. Nishioka Y, Yoshioka S, Kusunose M, Cui T, Hamada A, Ono M, *et al.* Effects of extract derived from *Eriobotrya japonica* on liver function improvement in rats. Biol Pharm Bull 2002;25:1053-7.
20. Huang Y, Li J, Cao Q, Yu SC, Lv XW, Jin Y, *et al.* Anti-oxidative effect of triterpene acids of *Eriobotrya japonica* (Thunb.) Lindl. Leaf in chronic bronchitis rats. Life Sci 2006;78:2749-57.
21. Ge JF, Wang TY, Zhao B, Lv XW, Jin Y, Peng L, *et al.* Anti-inflammatory effect of triterpenoic acids of *Eriobotrya japonica* (Thunb.) Lindl. Leaf on rat model of chronic bronchitis. Am J Chin Med 2009;37:309-21.
22. Hamada A, Yoshioka S, Takuma D, Yokota J, Cui T, Kusunose M, *et al.* The effect of *Eriobotrya japonica* seed extract on oxidative stress in adriamycin-induced nephropathy in rats. Biol Pharm Bull 2004;27:1961-4.
23. Wu G, Chai J, Suber TL, Wu JW, Du C, Wang X, *et al.* Structural basis of IAP recognition by SMAC/DIABLO. Nature 2000;408:1008-12.
24. Siegelin MD, Reuss DE, Habel A, Herold-Mende C, von Deimling A. The flavonoid kaempferol sensitizes human glioma cells to TRAIL-mediated apoptosis by proteasomal degradation of survivin. Mol Cancer Ther 2008;7:3566-74.
25. Kim MS, You MK, Rhuy DY, Kim YJ, Baek HY, Kim HA, *et al.* Loquat (*Eriobotrya japonica*) extracts suppress the adhesion, migration and invasion of human breast cancer cell line. Nutr Res Pract 2009;3:259-64.
26. Khan AW, Kotta S, Ansari SH, Sharma RK, Ali J. Potentials and challenges in self-nanoemulsifying drug delivery systems. Expert Opin Drug Deliv 2012;9:1305-17.
27. Government of India, Controller of Publications. The Ayurvedic Pharmacopeia of India. 1st ed., Part. 1. Vol. 2. Apx. 3. New Delhi: Government of India, Controller of Publications; 1999. p. 190.
28. Government of India, Controller of Publications. The Ayurvedic Pharmacopeia of India. 1st ed., Part. 1. Vol. 2. Apx. 3. New Delhi: Government of India, Controller of Publications; 1999. p. 191.
29. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 42nd ed., Vol. 6. Pune: Nirali Prakashan; 2009. p. 16-8.
30. Liang ZZ, Aquino R, Feo VD, Simone FD, Pizza C. Polyhydroxylated triterpenes from *Eriobotrya japonica*. Planta Med 1990;56:330-2.
31. Reich E, Schibli A. Stationary phases for planar separations – Plates for modern TLC. LC GC N Am 2005;23:58-69.
32. Tyszczuk-Rotko K, Wójciak-Kosior M, Sowa I. Voltammetric determination of betulinic acid at lead film electrode after chromatographic separation in plant material. Anal Biochem 2013;436:121-6.
33. Vyas M. Nutritional profile of spinach and its antioxidant & antidiabetic evaluation. Int J Green Pharm 2017;11:192.

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