

# Phyto constituents screening of loquat leaf extract using different solvents and antioxidant potential assessment

D. Kasthuri Santira Kumari, S. Iruthaya Kalai Selvam\*

Kumari DKS, Selvam SIK. Phyto constituents screening of loquat leaf extract using different solvents and antioxidant potential assessment. *AGBIR.2023;39(4):578-580.*

The presence of phytochemicals or the secondary metabolites in the plants are responsible for the medicinal properties of the plants. The phytochemicals are the bioactive component which promotes the physiological actions and enhances the immune system and protects the body from harmful pathogens. The phytochemical analysis is carried out to examine and investigate the presence of active chemical constituents such as the phenolic compounds, flavanoids, tannins and alkaloids as they play vital role in treating some

destructive diseases. Loquat is a subtropical plant with lot of medicinal values. Different extracts of loquat leaf with different solvents were used for phytochemical analysis. The purpose of the study is to find out the impact of the extract with different solvents and their phenolic, flavanoids, alkaloids and terpenoids content and their antioxidant potential. The secondary metabolites were found to be present in all the extracts. The different extracts showed effective anti-oxidant property.

**Key Words:** *Phytochemical screening; Loquat leaf extract; Eriobotrya japonica; Antioxidant potential*

## INTRODUCTION

The chemical substances that are naturally occurring in the plants are called phytochemicals. The secondary metabolites phenols, tannins, flavonoids, saponins and alkaloids are those which reveal the medicinal and physiological activities. Medicinal plants are of great importance for the health of the individual and communities [1-3]. The medicinal property of the plants is due to the presence of the bio active compounds. The first step is the phytochemical screening in processing a plant based drug [4]. The plant loquat is Cultivated in various parts of the world and the plant possess Pharmacological activities including anti-oxidant, anti-cancer and anti-hyperglycemic activity [5]. When compared with the pharmaceutical drugs it has been widely reported that the natural products are more effective [6].

In the traditional Chinese medicine, the loquat is widely playing an important role and used in ancient times to treat various diseases like acute bronchitis, cough and asthma. Research shows that loquat leaves extract possess many anti-oxidant, and different extract exhibit bioactivity which helps in treating inflammation, diabetes, cancer, microbial infection and other health issues along with aging, pain and allergy. The isolated phenolics and terpenoids were characterized to give a better understanding of chemical mechanisms of loquat leaf extract's bioactivities [7,8].

Eriobotrya japonica which is commonly called as Loquat belongs to the family Rosaceae. E. japonica is native to China; however it is cultivated in many countries like, India, Japan, South Africa and America. It is believed that combating and preventing disease is due to the presence of the effective phytochemicals present in the plants and the antioxidant properties they possess in them [9].

## MATERIALS AND METHODS

### Collection of plant material

The loquat leaves were collected from Bodi which is located in Theni District. The collected leaves were dusted and cleaned with running water, then dried at room temperature. The dried leaves were then chopped and grounded into a fine powder and used for preparation of the extract.

### Preparation of the sample for extraction

The finely grounded powder of E. japonica leaves were taken and about 100 gms powder was macerated with 500 ml of each solvent (Ethanol, methanol and distilled water) for about 48 hours at the room temperature. The extracts

were then filtered using Whatman no.1 filter paper and then using a rotary evaporator the solvents were evaporated. The extracts were stored at 4°C for further use.

### Qualitative analysis for phytochemicals

Phytochemical screening tests on the Aqueous (simple leaf extract), Ethanolic, methanolic extracts were done to determine the phytochemical contents in each in terms of quality following the method explained by Harborne [10]. The plant's medical properties are due to their phytochemical components, which have specific pharmacological effects on the human body refers Akinmoladun [11].

**Phytochemical analysis:** The prepared extracts were tested for the presence of bioactive compounds using the standard techniques.

**Test for alkaloid:** The filterates were treated with Mayer's reagent (mercuric chloride and potassium iodide in water) the presence of alkaloids specified by the formation of yellow colored precipitates.

**Test for carbohydrates:** The filterates were treated with naphthol and sulphuric acid and the presence of carbohydrate was noted by the appearance of violet color.

**Molisch's test:** The filtrates were treated with two drops of alpha naphthol solution, and drop wise conc. sulphuric acid was carefully dropped using dropper along the sides of the test tubes, the presence of carbohydrate was confirmed by the formation of violet color at the junction of the interface of two liquids.

**Test for glycosides:** About 5 ml of each extract was shaken for 15 minutes with 5 ml of distilled water and the formation of foam showed the presence of glycosides.

### Test for phytosterols

**Salkowski's test:** The plant extracts were mixed with chloroform, then about 5-6 drops of conc. sulphuric acid was added to each filtrate and shaken gently and allowed to stand carefully. The presence of triterpens (phytosterol) was indicated by the appearance of golden yellow colour.

### Test for flavonoids

**Alkaline reagent test:** The plant extracts were treated with 2-3 drops of sodium hydroxide solution, the acute yellow colour formation indicated the

Department of Zoology, Jayaraj Annappaikiam College for Women (Autonomous), Periyakulam, Theni District, Affiliated to Mother Teresa Women's University, Kodaikanal, Tamil Nadu, India

**Correspondence:** S. Iruthaya Kalai Selvam, Department of Zoology, Jayaraj Annappaikiam College for Women (Autonomous), Periyakulam, Theni District, Affiliated to Mother Teresa Women's University, Kodaikanal, Tamil Nadu, India, E-mail: [kasthuriharison@gmail.com](mailto:kasthuriharison@gmail.com); [kalai.akila@gmail.com](mailto:kalai.akila@gmail.com)

**Received:** 05-May-2023, Manuscript No. AGBIR-23-97920; **Editor assigned:** 08-May-2023, Pre QC No. AGBIR-23-97920 (PQ); **Reviewed:** 29-May-2023, QC No. AGBIR-23-97920; **Revised:** 08-Jun-2023, Manuscript No. AGBIR-23-97920 (R); **Published:** 15-Jun-2023, DOI:10.35248/0970-1907.23.39. 578-580



This open-access article is distributed under the terms of the Creative Commons Attribution Non-Commercial License (CC BY-NC) (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits reuse, distribution and reproduction of the article, provided that the original work is properly cited and the reuse is restricted to noncommercial purposes. For commercial reuse, contact [reprints@pulsus.com](mailto:reprints@pulsus.com)

presence of flavanoids, and by the addition of some drops of sulphuric acid changed it to colourless.

Test for phenols and tannin: About 3-4 drops of 0.1%v/v ferric chloride was added to the filtered samples and the change of colour to brownish green, indicated the presence of phenols or the tannins.

**Anti-oxidant assay**

Assessment of antioxidant activity using 1,1 Diphenyl 2 Picryl Hydrazyl (DPPH) radical scavenging activity. Plant extracts with different concentration were taken (20-100 µl/mg) was prepared from the stock solutions. Approximately 200 µl of DPPH was mixed with 100 µl of extract solutions and they were incubated for 30 minutes in the dark condition under room temperature for 72 hours. Ascorbic acid was used as standard and the absorbance was measured at 517 nm using UV-vis spectrophotometer. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenging Activity (\%)} = \frac{\text{A Control} - \text{A test}}{\text{A Control}} \times 100$$

**RESULTS AND DISCUSSION**

**Making of the extract**

The maceration of loquat leaf is done with various solvents like ethanol, methanol, and distilled water. The samples were kept for 72 hours and using the rotary evaporator the sample was obtained in a thick condensed form.

**Screening for phytochemistry**

Qualitative phytochemical Screening of loquat leaf extract as simple leaf extract, methanolic extract, ethanolic extract were analyzed to identify the presence of the secondary metabolites was performed and the results were presented in the Table 1. All the extracts showed the presence of the secondary metabolites.

**TABLE 1**  
**Phytochemical screening results of loquat leaf extract**

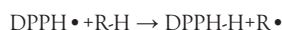
Phytochemicals	Extracts		
	Simple leaf extract	Ethanol	Methanol
Carbohydrates	+	+	+
Proteins	+	+	+
Phenol, tannin	+	+	+
Alkaloids	+	+	+
Flavanoids	+	+	+
Saponins	+	+	+
Sterols	+	+	+

**Note:** (+)=Presence, (-)=Absence. Here the secondary metabolites are noted (+) which indicates their presence.

**Anti-oxidant assay-DPPH assay**

Anti-oxidants are the substances which has the ability to inhibit or prevent oxidation on substrates which is caused by the free radicals [12].

Antioxidant analysis is the process which measures the ability of the substance to scavenge the free radicals.

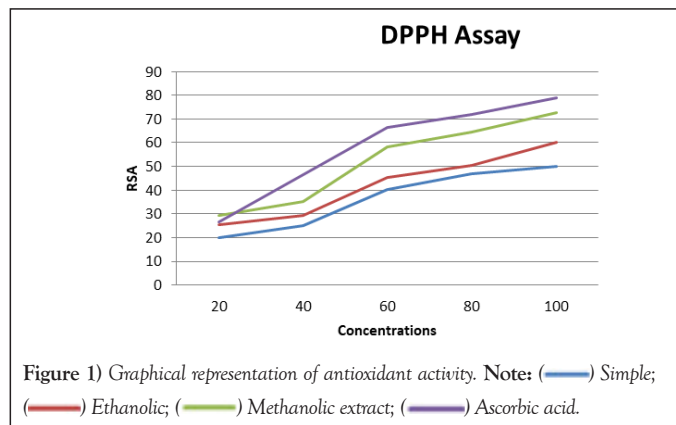


Where DPPH• is the DPPH free radical, R-H is the sample (antioxidant), DPPH-H is the reduced form of DPPH, and R• is the oxidized form of the sample.

Antioxidants react with the 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) which stabilizes free radicals and reduced DPPH. DPPH will react with hydrogen atom present in the radical reducer compounds which forms more stable 1,1-Diphenyl-2-Picrylhydrazine (DPPH-H). DPPH reagent while reacting with the antioxidants changes the colour from purple to yellow. Colour intensity or the degree of colour depends on the antioxidants ability of the sample [13].

The free radical scavenging activity for the studied extracts depends upon

the dosage. IC<sub>50</sub> value was calculated with the dose response curve (Figure 1).



The methanol and ethanol extracts have the IC<sub>50</sub> nearly same 3.06 and 3.8.

The simple leaf extract and the standard Ascorbic acid have the IC<sub>50</sub> as 2.5 and 2.7 and are effective. As the IC<sub>50</sub> value is low its said to be effective and prevents cell damages.

To compare the effectiveness of different antioxidants, IC<sub>50</sub> and R<sup>2</sup> values obtained from antioxidant assays can be used. The R<sup>2</sup> value, also known as the coefficient of determination, is a statistical measure of how well the antioxidant assay data fits the linear regression model. A higher R<sup>2</sup> value indicates a better fit between the data and the linear regression model, suggesting that the assay results are more reliable and accurate.

A study by Zhao et al., [14] compared the antioxidant activity of different tea extracts using both IC<sub>50</sub> and R<sup>2</sup> values. It says that green tea extract had the lowest IC<sub>50</sub> value, indicating the strongest antioxidant activity, while black tea had the highest IC<sub>50</sub> value, indicating the weakest antioxidant activity. In addition, the R<sup>2</sup> values for the green tea extract were higher than those for the black tea extract, suggesting that the data for green tea was more reliable and accurate [14].

According to Mogole the methanol extract showed more scavenging activity [15].

The antioxidant activity of the extracts against various concentrations is shown in Table 2.

**TABLE 3**  
**Anti-oxidant activity of the extracts of loquat leaf showing percentage of inhibition with various concentrations**

Sample (µl/mg)	Percentage of inhibition (%)			
	Simple leaf extract	Ethanolic extract	Methanolic extract	Ascorbic acid
20	20.1	25.3	29.38	26.55
40	25.2	29.43	35.33	46.53
60	40.1	45.42	58.24	66.49
80	47.05	50.45	64.59	71.82
100	50.12	60.21	72.78	78.76

The reduction potential of the different extracts of loquat leaf and IC<sub>50</sub> is shown in Table 3.

**TABLE 2**  
**IC<sub>50</sub> values for Anti-oxidant activity of loquat leaf**

Sample (mg/L)	Percentage of inhibition (%)			
	Simple leaf extract	Ethanolic extract	Methanolic extract	Ascorbic acid
R <sup>2</sup>	0.9493	0.9691	0.9494	0.9271
IC <sub>50</sub>	2.5	3.802281	3.065792	2.749519

## CONCLUSION

It can be concluded that the source of secondary metabolites is present in the simple leaf extract, as well as in methanol and ethanol extract. Phytochemical results showed the presence of flavonoid, terpenoid, phenolic, alkaloid, and saponin compounds that function as antioxidants. The medicinal value of the plants which gives the healing potential is due to the presence of these flavonoids, alkaloids and terpenoids. During the assay, the DPPH free radical is reduced by the sample, which causes a colour change from purple to yellow. The degree of colour change is proportional to the amount of DPPH scavenged by the sample. Flavanoids has the ability to stabilize and delocalize unpaired electrons in free radicals by acting as a hydrogen donor.

IC<sub>50</sub> is a measure of the effectiveness of an antioxidant in inhibiting a specific biochemical reaction, such as the oxidation of a particular substrate. The IC<sub>50</sub> of an antioxidant can also be useful in developing antioxidant-based therapies for diseases. If an antioxidant has a low IC<sub>50</sub>, it may be more effective at preventing or reducing oxidative damage in cells and could be a potential candidate for treating diseases caused by oxidative stress.

Antioxidants are important because they can prevent or reduce damage caused by free radicals, which are molecules with unpaired electrons that can damage cells and contribute to the development of various diseases, such as cancer, diabetes, and heart disease. Antioxidants work by donating an electron to neutralize the free radical, preventing it from damaging cells.

The IC<sub>50</sub> value provides a measure of the concentration of the antioxidant required to inhibit 50% of the reaction being studied. A lower IC<sub>50</sub> value indicates greater antioxidant activity, as it requires less of the antioxidant to achieve the desired level of inhibition.

The R<sup>2</sup> value, also known as the coefficient of determination, is a statistical measure of how well the antioxidant assay data fits the linear regression model. A higher R<sup>2</sup> value that is 0.9 nearly 1 indicates a better fit between the data and the linear regression model, suggesting that the assay results are more reliable and accurate. Therefore, by using both IC<sub>50</sub> and R<sup>2</sup> values, we have obtained a comprehensive understanding of the antioxidant effectiveness of different compounds and make informed decisions about which antioxidants may be most beneficial for preventing or treating diseases associated with oxidative stress. Overall, the IC<sub>50</sub> of antioxidants is an important parameter in understanding the mechanism of action and efficacy of antioxidants and can guide the development of therapeutic interventions for diseases associated with oxidative stress.

## REFERENCES

1. Yadav R, Khare RK, Singhal A. Qualitative phytochemical screening of some selected medicinal plants of shivpuri district (MP). *Int J Life Sci Scient Res*. 2017; 3(1):844-847.
2. Grover N, Patni V. Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of *Woodfordia fruticosa* (L) Kurz. *Leaves*. *Int J Pharm Pharm Sci*. 2013; 5(4):291-295.
3. Ayoola GA, Coker HA, Adesegun SA, et al. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Trop J Pharm Res*. 2008; 7(3):1019-1024.
4. Islam T, Al Mamun A, Rahman H, et al. Qualitative and quantitative analysis of phytochemicals in some medicinal plants in Bangladesh. *JCBPS*. 2016; 6(2):530.
5. Ahumada J, Fuentealba C, Olaeta JA, et al. Bioactive compounds of loquat (*Eriobotrya japonica* Lindl.) cv. Golden Nugget and analysis of in vitro functionality for hyperglycemia management. *Ciencia e investigación agraria: revista latinoamericana de ciencias de la agricultura*. 2017; 44(3):272-284.
6. Sanni O, Erukainure OL, Chukwuma CI, et al. *Azadirachta indica* inhibits key enzyme linked to type 2 diabetes in vitro, abates oxidative hepatic injury and enhances muscle glucose uptake ex vivo. *Biomed Pharmacother*. 2019; 109:734-743.
7. Liu Y, Zhang W, Xu C, et al. Biological activities of extracts from loquat (*Eriobotrya japonica* Lindl.): a review. *Int J Mol Sci*. 2016; 17(12):1983.
8. Zhang W, Zhao X, Sun C, et al. Phenolic composition from different loquat (*Eriobotrya japonica* Lindl.) cultivars grown in China and their antioxidant properties. *Molecules*. 2015; 20(1):542-555.
9. Halliwell B. Free radicals, antioxidants and human disease. Where are we now? *J Lab Clin Med*. 1992; 119:598-620.
10. Harborne AJ. *Phytochemical methods a guide to modern techniques of plant analysis*. Springer science & business media; 1998.
11. Akinmoladun AC, Ibukun EO, Afor E, et al. Chemical constituents and antioxidant activity of *Alstonia boonei*. *Afr J Biotechnol*. 2007; 6(10).
12. Hasibuan FE, Syahfitri W, Ilyas S, et al. Phytochemical screening, antioxidant activity and thin-layer chromatography test of methanol extract and simplicia leaves of loquat (*Eriobotrya japonica* Lindl). *IOP Conf Ser: Mater Sci Eng*. 2020; 012069.
13. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J Sci Technol*. 2004; 26(2):211-219.
14. Zhang J, Shen X, Wang K, et al. Antioxidant activities and molecular mechanisms of the ethanol extracts of *Baccharis propolis* and *Eucalyptus propolis* in RAW64. 7 cells. *Pharm Biol*. 2016; 54(10):2220-2235.
15. Mogole L, Omwoyo W, Mtunzi F. Phytochemical screening, anti-oxidant activity and  $\alpha$ -amylase inhibition study using different extracts of loquat (*Eriobotrya japonica*) leaves. *Heliyon*. 2020; 6(8):e04736.