

ORIGINAL ARTICLE

Integrative Assessment of Antioxidant and Anti-inflammatory Activities of *Syzygium cumini* and *Eriobotrya japonica*

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ABSTRACT

Medicinal plants which include multiple types of phytonutrients that have positive effects for human health have attracted greater attention in recent decades. Many secondary metabolites from numerous plant species have been exploited as therapeutic agents for managing an assortment of human illnesses since ancient times. There are reports of secondary metabolites' historic medicinal uses all across the world. In view of this, the objective of our study is to analysed the phytochemical content of hydroethanolic leaves extract using standard procedure along with the antioxidant (phenolic content and vitamin estimation i.e. C and E) and anti-inflammatory (protein denaturation assay) activity of *Syzygium cumini* and *Eriobotrya japonica*. The results of these studies showed that *Syzygium cumini* having higher amount of antioxidant (phenolic content and vitamin C & E) and anti-inflammatory activity as compared to *Eriobotrya japonica*. Based on these data, it appears that both plants are plentiful with biologically active substances that might be used to create antioxidant and anti-inflammatory agents. Additional investigation into the phytochemical characteristics of these plants might help develop new medications to treat tropical illnesses while lowering the need for artificial chemicals, so preventing environmental damage.

Key words: *Syzygium cumini*; *Eriobotrya japonica*; antioxidant; anti-inflammatory

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INTRODUCTION

The northern part of the Himalayan region of India (Jammu and Kashmir) is home to a broad inventory of medicinal plants owing to its subtropical and alpine climatic zones. In the literature, researchers reported more than 1,500 types of medicinal plants in the geographic area; the majority of those medicinal plants have been applied in medicine (folk, Ayurvedic, and Unani) [1]. Those plants with medicinal benefits and great economic value include *Saussurea costus* (kuth), *Podophyllum hexandrum* (Indian mayapple), etc. Not only are these medicinal plants so essential to the pharmaceutical and herbal industries, but they also enhance the quality of life for local citizens. These medicinal plant species are important not just for their health benefits but also for their ecological and economic effects [2, 3]. We studied two nutrient-dense, medicinally necessary fruits with several health benefits: *Eriobotrya japonica* (loquat) and *Syzygium cumini* (black plum or jamun). These two plants have been used traditionally in many different medical systems, and scientific research has lately looked into their potential as drugs.

The leaves of *Syzygium cumini* are known to contain β -sitosterol, n-hentriacontane, noctacosanol, n-triacontanol, n-dotriacontanol, quercetin, myricetin, myricitrin and the flavonol glycosides myricetin 3-O-(4"-acetyl)- α -L-rhamnopyranosides, acylated flavonol glycosides. Commonly known as jamun, black plum, or Indian blackberry, *Syzygium cumini* is a tropical evergreen tree that is well-known for its therapeutic properties in both conventional and alternative medicine [3]. Members of the *Myrtaceae* family, this plant has long been utilized in Siddha, Ayurveda, and Unani medicine to cure a variety of illnesses. Numerous bioactive substances, such as flavonoids, anthocyanins, tannins, alkaloids, and the special alkaloid jamboline, which correlates to its hypoglycemic qualities, are found in *Syzygium cumini*'s fruit, seeds, bark, and leaves. The seeds' antidiabetic qualities are especially well-known since they increase insulin

sensitivity and aid in blood sugar regulation [2-6]. The stem bark contains friedelin, friedelan-3- α -ol, betulinic acid, β -sitosterol, kaempferol, β -sitosterol-D-glucoside, gallic acid, ellagic acid, gallotannin and ellagitannin and myricetin (Fig.1). The flowers are observed to contain oleanolic acid, ellagic acids, isoquercetin, quercetin, kampferol and myricetin. The anthocyanins, delphinidin, petunidin, malvidin-diglucosides, are present in the pulp of fruit and responsible for their bright purple color [4-7].

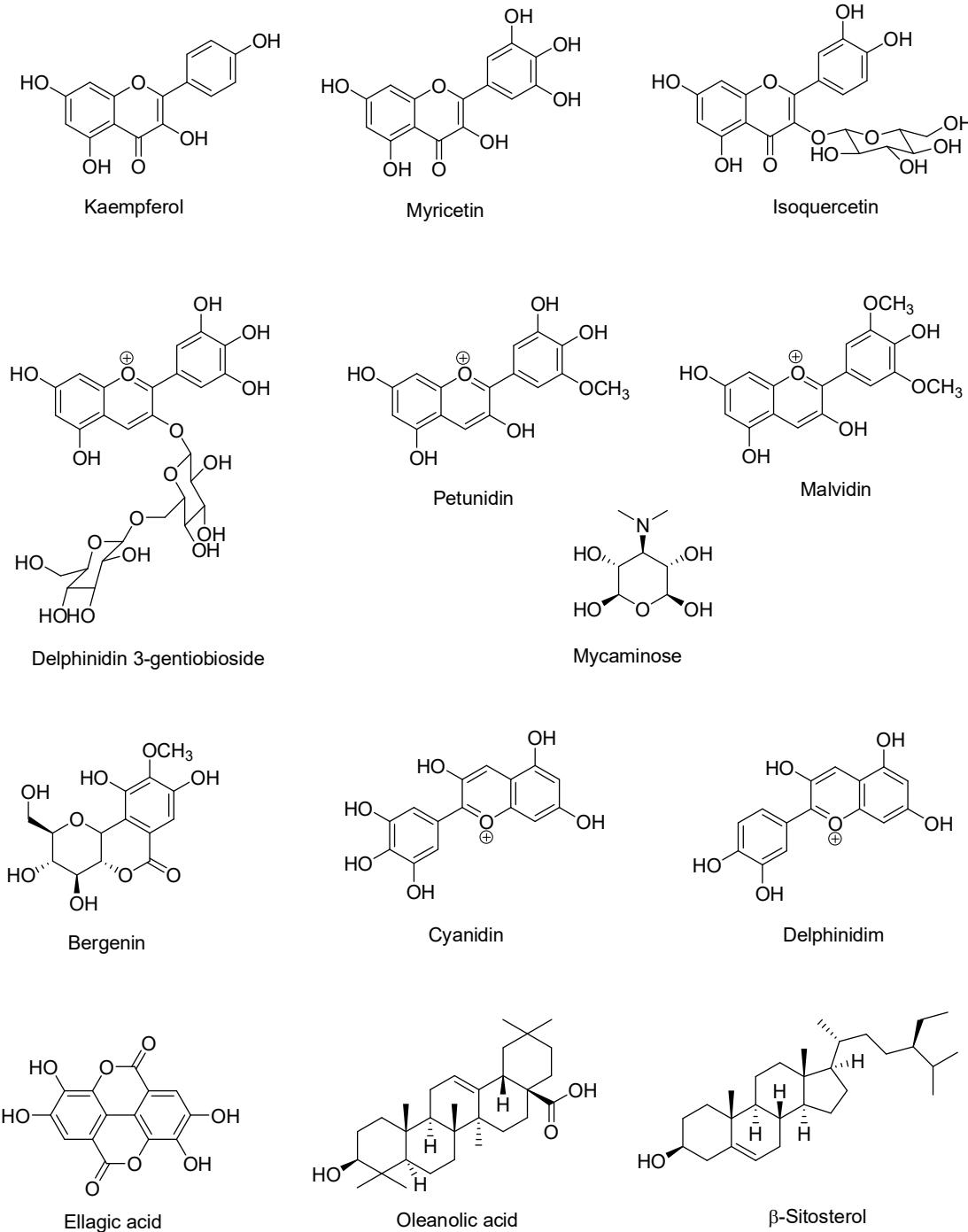


Fig. 1. Representative phytochemicals present in various parts of *Syzygium cumini*

The agriculture commodity loquat, or *Eriobotrya japonica*, has become extremely popular in the Jammu region in northern India because of its capability to adapt to both subtropical and temperate climates, according to reports. Because of its steep topography and temperate temperature, loquats may be grown there, particularly in places that receive plenty of sunlight and drainage [8-10]. The plant has important therapeutic uses in indigenous cultures along with its dietary benefits. *Eriobotrya japonica* leaf has anti-inflammatory, antioxidant, and antitussive (cough-relieving) properties, thus it should be used often in herbal medicine. Leaf extracts are frequently utilized in decoctions or teas for relieving respiratory

problems such as bronchitis, asthma, and coughs [9-11]. Additionally, they are thought to help regulate blood sugar levels and the functioning of the liver (Fig.2).

In contrast, instrumental techniques have identified over 160 phytochemicals in the leaves of *Eriobotrya japonica*, including triterpenes, flavonoids, sesquiterpene glycosides, phenylpropanoids, megastigmane derivatives, and organic acids. Additionally, about 169 volatile compounds, such as farnesyl acetate, n-hexadecanoic acid, (+)-carvone, and (E)-nerolidol, have been documented. Key triterpenes found in the leaves - like euscaphic acid, tormentic acid, corosolic acid, ursolic acid, oleanolic acid, and betulinic acid - are recognized for their anti-inflammatory, antiviral, and antitumor potential. The leaves also contain sesquiterpene glycosides such as Loquatifolin A, Citroside A, and various Vomifolol isomers, which have demonstrated antihyperglycemic effects. Diterpenes like phytol offer a wide range of biological benefits, including antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. Among the notable flavonoids present are quercetin, kaempferol, catechin, (-)-epicatechin, (+)-gallocatechin, and hesperidin.¹²⁻¹⁴ Phenylpropanoid compounds like liguersinol, eriobotrin, and ferulic acid, along with organic acids such as citric, malic, gallic, and chlorogenic acids, aldehydes such as Vanillin, Protocatechuic aldehyde and fatty acids such as Tricosanoic acid further contribute to the plant's medicinal profile. Recent studies have highlighted the potential of certain bioactive constituents - especially flavonoids, triterpenoids, and tannins found in loquat leaves and seeds—to support the management of type 2 diabetes. Compounds like cinchonain-Ib, thymosaponin, chlorogenic acid, and epicatechin have shown efficacy in lowering blood glucose levels, improving lipid profiles, enhancing insulin sensitivity and secretion, and promoting better glucose tolerance. The fruit is also utilized for boosting immune and gastrointestinal health because to its high vitamin A, B, and C content. Because the seeds contain possible toxic compounds, it should be thoroughly treated before consumption [12-14]. In this work, we looked over the phytochemical content including an antioxidant characteristic of the hydroethanolic leaf extract from *Syzygium cumini* and *Eriobotrya japonica*.

MATERIAL AND METHODS

Collection of plant material

Following collection, plant materials (*Syzygium cumini* and *Eriobotrya japonica* leaves) must be cleaned to remove contaminants before getting shade-dried or oven-dried at monitored temperatures so as to avoid the active ingredient breakdown. Usually, the dried leaves are pounded into a fine powder then preserved in sealed plastic bags in an area that is dry and cool until we need them again. Making sure traceability requires precise labelling that includes the date, place, and collector's name.

Preparation of hydroethanolic extract

After meticulously weighing the pulverized leaves, these were then blended with a hydroethanolic solution, which usually combines ethanol and water in the proper ratio (for instance, 70:30 v/v), for creating the hydroethanolic extract. The combination of ingredients was constantly stirred or agitated around ambient temperature over a predetermined period of time, often 24 to 72 hours. After extraction, the solution underwent filtering to remove any remaining plant material, and the resulting filtrate was collected. The rotary evaporator was then utilized for eliminating the solvent from the mixture at a lower pressure, producing a crude hydroethanolic extract that was stored at 4°C until it was required.

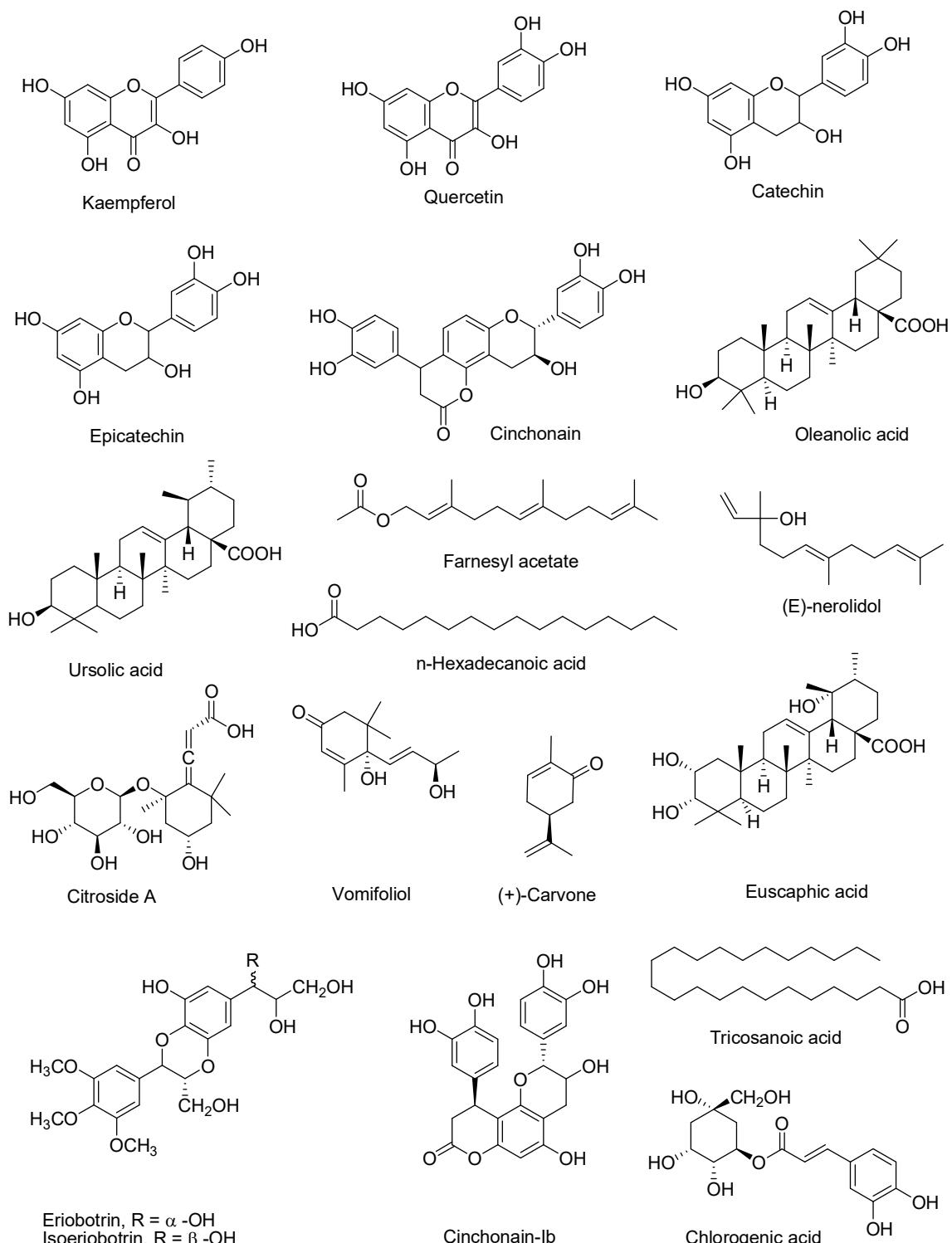


Fig. 2. Representative phytochemicals present in various parts of *Eriobotrya japonica*

Qualitative analysis of phytochemicals

Qualitative phytochemical examination revealed the presence of major secondary metabolites, including flavonoids, terpenoids, alkaloids, and tannins. Various standard tests were employed for every kind of content [15]. A few drops of mild ammonia solution combined with strong sulfuric acid were added to the resultant mixture (hydroethanolic leaves extract of *Syzygium cumini* and *Eriobotrya japonica*) to treat flavonoids; the appearance of a yellow hue indicated a successful reaction. By applying concentrated sulfuric acid, which results in a reddish-brown coloration at the contact, terpenoids were identified using the Salkowski test. Using Mayer's and Wagner's reagents, alkaloids were found; a positive test culminated

in a creamy or reddish-brown precipitate. By adding ferric chloride solution to the hydroethanolic leaves extract, which produces a blue-black or greenish-black colouring, the presence of tannins was confirmed.

Estimation of phenolic content

The Folin-Ciocalteu examination was implemented for using spectroscopy quantify all of the phenolic compounds in the hydroethanolic leaf extract of *Syzygium cumini* and *Eriobotrya japonica*, using gallic acid as a reference. Three milliliters of distilled water, 0.25 milliliters of Folin-Ciocalteu reagent, and 0.5 milliliters of hydroethanolic leaf extract were combined to create the reaction mixture, which was then shaken. Following a 5-minute dark period, 1 milliliter of 7.5% Na₂CO₃ was added, and the mixture was then allowed to sit at room temperature for 90 minutes. In addition, a reagent blank was created using distilled water. The absorbance has been determined at 760 nm employing a dual beam UV/Vis spectrophotometer in comparison to the produced reagent blank. The extract's total phenolic component content has been determined in milligrams of gallic acid equivalent (GAE) per 100 grams of sample (mg GAE/100 g). Three separate analyses were performed on each sample [16].

Anti-inflammatory activity (Denaturation assay)

The sample's anti-denaturation activity was determined using a BSA test. To put it briefly, the reaction mixture consisted of BSA solution and varying concentrations of *Syzygium cumini* and *Eriobotrya japonica* which were created in an appropriate solvent. The solutions were incubated for 20 minutes at 37°C after the pH had been adjusted to 6.3 employing hydrochloric acid (HCl). Following incubation, the samples were heated to 57°C for an additional twenty minutes in order to denature the proteins. The turbidity was determined employing spectrophotometry on 660 nm after cooling. The fraction of decrease in protein denaturation was evaluated in comparison to a control sample, and more significant inhibition indicated more anti-inflammatory effect [17].

Estimation of vitamins (C, and E)

To put it briefly, 20 milliliters of 0.4% oxalic acid were mixed separately with 1 gram of the hydroethanolic leaves extract of *Syzygium cumini* and *Eriobotrya japonica*. After filtering it via Whatman filter paper, 1 milliliter of the filtrate was combined with 9 milliliters of indophenol reagent. The solution's absorbance was measured at 520 nm. Plotting a standard curve had been performed following the same procedure was carried out to create the vitamin C standard at different concentrations.

20 milliliters of ethanol were added to 0.5 grams of the hydroethanolic leaves extract, and the mixture was shaken for 20 minutes to estimate the amount of vitamin E. After then, it was filtered. Following that, 1 milliliter of the filtrate was combined with 1 milliliter of 0.2% ferric acid in ethanol and 1 milliliter of 0.5% α -adipyrine. Five milliliters of distilled water were added to the solution. At 520 nm, the absorbance of the solution was measured. Similarly, the vitamin E standard was made at different concentrations and a standard curve was constructed.

RESULTS

Qualitative analysis of phytochemicals

The studies of hydroethanolic leaf extract of *Syzygium cumini* and *Eriobotrya japonica* reveal the presence of alkaloids, flavonoids, terpenoids, and tannins, which is determined qualitatively. **Table 1** reveals the existence of a respective secondary metabolite, which is determined qualitatively (colour formation).

Table 1. Phytochemical analysis (qualitatively)

Plant material	SECONDARY METABOLITES			
	Alkaloids	Flavonoids	Terpenoids	Tannins
<i>Syzygium cumini</i>	Yellowish green	Yellowish green	Light brown	White
<i>Eriobotrya japonica</i>	Greenish yellow	Yellow	Yellow	Greenish black (Catechol tannins)

Phenolic content

The hydroethanolic leaf extracts of *Syzygium cumini* and *Eriobotrya japonica* had the highest amount of total phenolic content (21.93 \pm 1.43 mg GAE/g) in the current investigation. The curve of calibration was computed by employing concentrations of gallic acid ranging from 1 to 5 mg/ml, and the findings were expressed as gallic acid equivalents (GAE). The findings are displayed in **Table 2**. Through intricate oxidation-reduction processes, several phenolic chemicals in the applied extract interact nonspecifically with the phosphotungstic and phosphomolybdic acids found in the Folin-Ciocalteu phenol reagent. Depending on how many phenolic groups they contain, different phenolic compounds respond differently to the Folin-Ciocalteu test.

Table 2. Phenolic content (mg/g gallic acid equivalent) of hydroethanolic leaves extract of *Syzygium cumini* and *Eriobotrya japonica*

Extracts	<i>Syzygium cumini</i>	<i>Eriobotrya japonica</i>
Total Phenolic content	24.92 ± 1.78	19.64 ± 1.32

Anti-inflammatory activity

The anti-inflammatory activity of *Syzygium cumini* and *Eriobotrya japonica* was evaluated using the BSA denaturation assay. The hydroethanolic leaves extract was tested at different concentrations, and its inhibitory effects were compared to standard values. The results showed a percentage of inhibition at 100 mg/ml. These values indicate that the *Syzygium cumini* and *Eriobotrya japonica* exhibits significant anti-inflammatory activity by inhibiting bovine serum albumin denaturation. Moreover, the anti-inflammatory properties of the leaf extracts were comparable to the standard diclofenac sodium at all tested concentrations as shown in **Fig.3**.

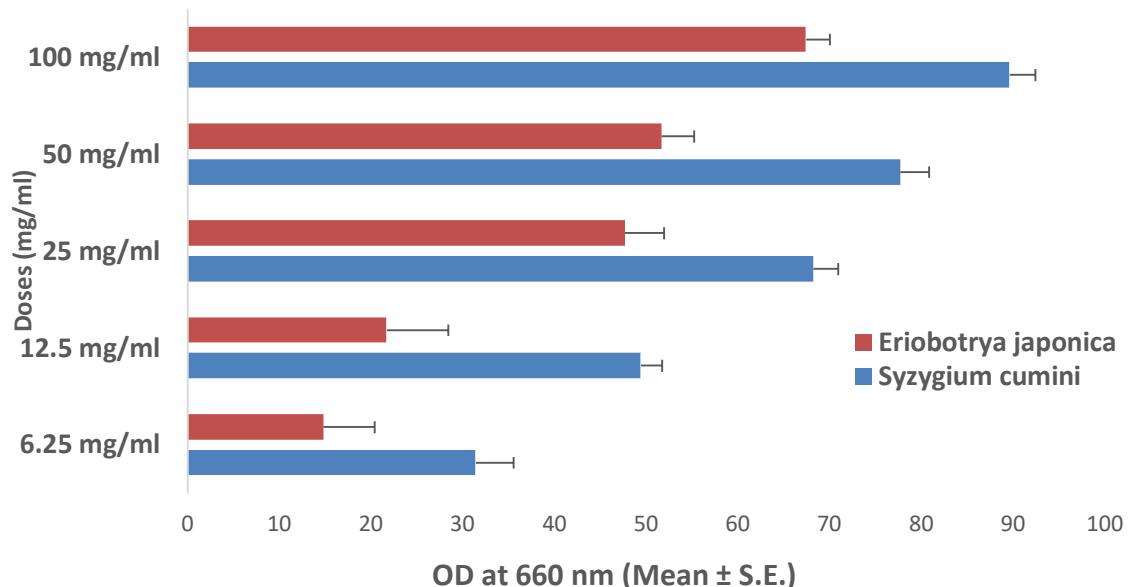


Fig.3. Anti-inflammatory activity of *Syzygium cumini* and *Eriobotrya japonica* using the protein BSA denaturation assay. Values were expressed in the form of Mean ± S.E. Statistical analysis (*P<0.05, **P<0.01 and ***P<0.001) was also performed using one-way ANOVA test.

Estimation of vitamins (C and E)

The effect of hydroethanolic leaf extract of *Syzygium cumini* and *Eriobotrya japonica* is shown in **Fig.4**. These studies indicated that *Syzygium cumini* contains a higher content of vitamin C and vitamin E as compared to *Eriobotrya japonica*. In this study, we used two standards (vitamin C and E) purchased from the market and prepared the standard curve, and readings should be calculated in the form of $\mu\text{g}/\text{ml}$.

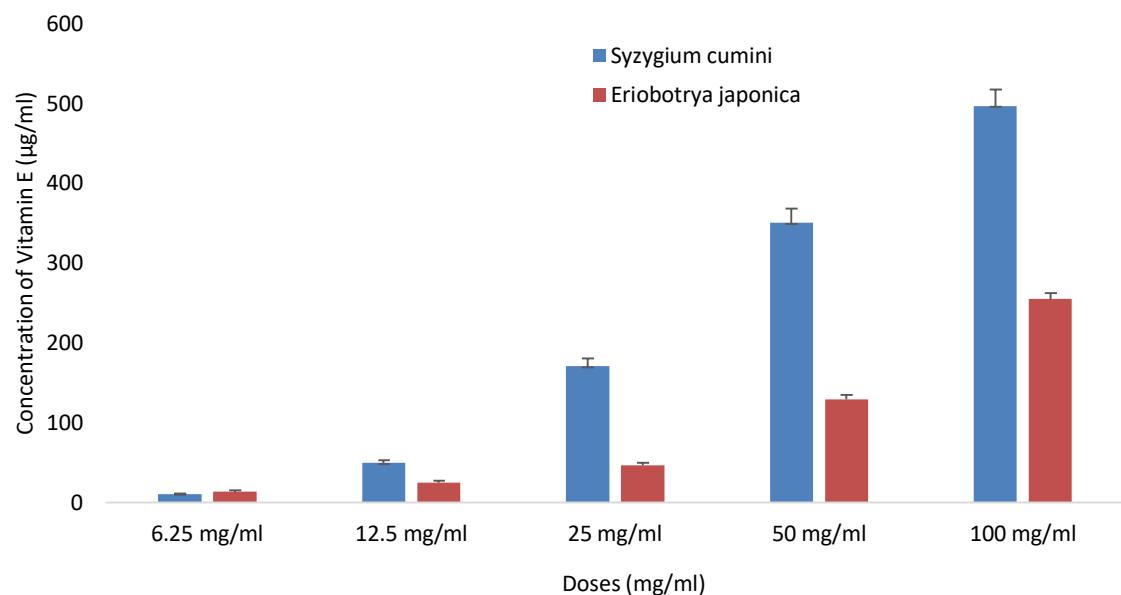
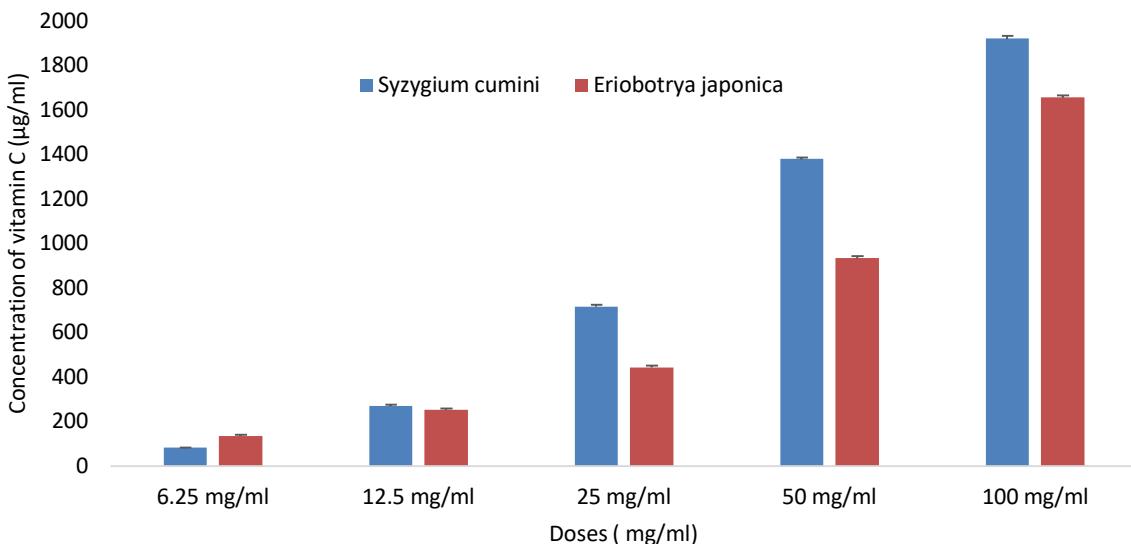


Fig.4. Estimation of vitamin C and E using variable concentration of *Syzygium cumini* and *Eriobotrya japonica*. Values were expressed in the form of Mean \pm S.E. Statistical analysis (*P<0.05, **P<0.01 and ***P<0.001) was also performed using one-way ANOVA test.

DISCUSSION

Research on the immunopharmacology of botanicals that could only be used in conventional medicine was conducted. The biological characteristics of *Eriobotrya japonica*, such as its evergreen status, massive leathery leaves, and fragrant white flowers that bloom in the winter, have been highlighted in literature. Similarly, *Syzygium cumini* is a large evergreen and densely foliaceous tree that produces small purple plums and is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol, and myricetin. The fruit is very rich in vitamins, minerals, and antioxidants and is frequently eaten raw or processed into syrups and jams. Traditional medicinal systems, especially those from East Asia, have documented utilizing it for the treatment of diabetes, respiratory issues, and ailments connected to inflammation. Recent research on drugs supports these uses by demonstrating antioxidant, antidiabetic, antiviral, or anti-inflammatory qualities [7-9]. Consequently, *Syzygium cumini* and *Eriobotrya japonica* continue to be topics that are intriguing in ethnopharmacology and natural product research, with increasing focus on their potential for creating nutritional supplements or herbal formulations. In this study, we found that hydroethanolic leaf extracts of *Syzygium cumini* and *Eriobotrya japonica* leaves contain

flavonoids, phenolic acids, and terpenoids, which exhibit strong antioxidant effects. These compounds scavenge free radicals and help reduce oxidative stress, contributing to the plant's use in managing chronic conditions like cardiovascular diseases and aging-related disorders. Studies have indicated that triterpenes and polysaccharides in *Syzygium cumini* and *Eriobotrya japonica* leaves can help regulate blood glucose levels by enhancing insulin sensitivity and reducing blood sugar spikes. In addition, leaves of *Syzygium cumini* are widely used in constipation and diabetes; fruits for pharyngitis and splenic diseases; and bark for astringents, anthelmintics, and carminatives. Furthermore, seeds are used as astringents and diuretics, especially in the treatment of diabetes [10-12]. In the present study, we focused on the hydroethanolic leaf extract of *Syzygium cumini* and *Eriobotrya japonica* and analyzed its anti-inflammatory activity against specific protein antigens.

Protein denaturation, which happens whenever an amino acid changes its native framework and function in biology, is highly associated with multiple kinds of inflammatory variables and has significant implications for inflammatory responses. The process of inflammation, an extensive and typical immunological mechanism caused by harm or infection requiring a number of molecular mechanisms, may be greatly aided by denaturation of proteins. In this study, extracts of *Syzygium cumini* and *Eriobotrya japonica* showed their inhibitory action against BSA. Denaturing proteins is one of the highest reported inflammation-causing factors. When there is an excessive buildup of fluid inside the cells, the RBC membrane ruptures, causing hemolysis. Red blood cells are more vulnerable to secondary harm when the membrane is damaged. Membrane stabilization can stop the leaking of serum protein and fluids into the tissue. An increase in membrane permeability serves as an intermediate step in this process, which is caused by inflammation.

Vitamin C could function as an antioxidant, offering an abundance of health-positive aspects while also participating in the stabilization of free radicals. Nevertheless, it can neutralize the majority of naturally occurring radicals and possesses an extensive array of antioxidant properties throughout the body. This extensive array of antioxidant properties contributes to various health benefits, including improved skin health, enhanced immune function, and reduced risk of chronic diseases. By effectively combating oxidative stress, vitamin C plays a crucial role in maintaining overall well-being. For overall well-being, it is essential to incorporate vitamin C-rich foods into our diet, such as citrus fruits, strawberries, and bell peppers. Regular intake can support not only physical health but also enhance mental clarity and vitality. It scavenges superoxide, nitroxide, hydroxide, and hydrogen peroxide while depleting vitamin E. In contrast, vitamin C is an antioxidant that exists by itself wholly and is generally found in fruits and vegetables. Vitamin C serves as a vital component for electron transport, hydroxylation workflows, and the oxidative degradation of aromatic chemicals in mammalian metabolism. According to reports, vitamin C decreases the likelihood of cancer and interacts significantly with other vitamins. Excessive vitamin A consumption, for example, is less hazardous to the body when vitamin C is present. Vitamin C is extremely important to humans; hence, its quantitative evaluation is growing increasingly crucial in many fields of analytical chemistry, including food and medicinal applications. Accurate measuring methods are crucial in these domains to guarantee product efficacy and quality. High-performance liquid chromatography (HPLC) and spectrophotometric analysis are prominent approaches to measuring vitamin C levels, providing for improved quality control and health evaluations. There is ongoing research into vitamin C's other roles, including its involvement in Michael addition reactions to generate conjugates of electrophilic intermediates and its significance in brain development and communication between cells through the production of hydrogen peroxide. These conjugates could be essential for a number of biological functions, including neuroprotection or cell communication.

To completely comprehend the consequences of these relationships in health and illness, more study is required. Comprehending these interplays might result in novel treatment approaches for ailments associated with oxidative stress and neurodegenerative illnesses. Researchers are interested in uncovering novel targets for intervention that improve health outcomes by gaining insight into the processes by which vitamin C regulates cellular functioning. Vitamin E is a necessary ingredient that scavenges lipid hydroperoxy radical to act as an antioxidant. High doses of vitamin E can interfere with redox cycling and/or vitamin K production, which can be advantageous for some people but harmful to others and this can affect blood coagulation. In our study, we found that both plants showed higher production of vitamin C as compared to vitamin E. In the literature, vitamin C and E supplementation trials that were carried out to lower LPO and oxidative stress have not been found to have a positive impact on clinical outcomes in illnesses linked to elevated oxidative stress levels. Patients with acceptable baseline vitamin levels are anticipated to encounter significant effects if disease development accelerates at low vitamin status. Vitamin level measurement would be especially crucial for individuals with lower baseline vitamin levels, such as hospitalized and elderly patients. The selection of endpoints, particularly those associated with the

aims of vitamin supplementation, is another crucial element influencing a clinical trial's outcome. In order to forecast appropriate dosage regimens for conclusive efficacy studies utilizing biomarker and clinical end points, this kind of data would offer informative data regarding dose-effect connections.

CONCLUSION

According to the current study, *Syzygium cumini* and *Eriobotrya japonica* have anti-inflammatory and antioxidant properties. These properties make them valuable in the development of natural remedies for various inflammatory conditions. Furthermore, their rich phytochemical profiles contribute to their potential health benefits, warranting further research into their medicinal applications. These applications could lead to innovative treatments for ailments such as arthritis, asthma, and other chronic diseases characterised by inflammation. Additionally, understanding the mechanisms behind their effects may facilitate the discovery of new therapeutic agents derived from these plants. These results are consistent with the need for natural antioxidants in medicines, nutraceuticals, and functional foods.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Permission to reproduce material from other sources

Not Applicable

Author Contributions

Dr Amit Gupta created concept and supervised study, Harleen Kour, Manvi Trogia, Riya Kohli, Ritumbhra Rajput and Akshay Kumar Sharma conducted the lab experiment, Dr Amit Gupta drafted the manuscript, Dr Surinder Paul Sharma reviewed the manuscript.

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