IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 11, May 2026
Impact Factor: 8.2

Pharmacognostical Screening and Evaluation of In Vitro Anti-Glycation Activity of Eucalyptus globulus Leaf Extract

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Abstract: *The non-enzymatic interaction between reducing sugars and proteins, lipids, or nucleic acids produces advanced glycation end products (AGEs), which accelerate the development of chronic conditions including diabetes, heart disease, and aging-related problems. In this work, the antiglycation efficacy of Eucalyptus globulus leaf extract was assessed, and the phytochemical components responsible for its therapeutic actions were examined. Eucalyptus globulus leaves were gathered, dried, pulverized, and extracted using the Soxhlet extraction technique with methanol and ethanol as solvents. While determining physicochemical characteristics including ash value and drying loss, preliminary phytochemical screening was carried out to find beneficial chemicals. An in vitro titration approach utilizing fructose-protein reaction mixtures at various extract concentrations (20–100 µg/mL) was used to assess antiglycation activity.*

Important secondary metabolites, such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic chemicals, were found during phytochemical screening, suggesting significant therapeutic potential. The crude drug's acceptable quality and moisture content were confirmed by the ash value and drying loss of 5% and 10%, respectively. The antiglycation test showed concentration-dependent inhibition, with glycation reactions being considerably decreased as extract concentration increased. At a dose of 100 µg/mL, the maximum antiglycation activity of 50% inhibition was noted. Because of their antioxidant qualities, phytoconstituents—especially flavonoids and phenolic compounds—may have a useful role in the decrease of titration volume and glycation end-product production. According to the study's findings, Eucalyptus globulus has a good antiglycation potential and might be used as a natural medicinal source to prevent glycation-related problems connected with aging and diabetes...

Keywords: *advanced glycation end products*

I. INTRODUCTION

Reducing sugars combine with proteins, lipids, or nucleic acids in a non-enzymatic biochemical process called advanced glycation, which produces toxic substances called advanced glycation end products (AGEs). Numerous chronic illnesses, including diabetes mellitus, cardiovascular problems, neurodegenerative diseases, renal dysfunction, and aging-related issues, are closely linked to the formation of AGEs. AGEs interfere with normal physiological processes by causing oxidative stress, inflammation, and cellular damage. As a result, glycation inhibition or prevention has drawn a lot of interest in biological and pharmacological research.

Because of their rich phytochemical composition, safety, and affordability, natural medicinal plants have been extensively investigated as alternative sources of therapeutic chemicals. Flavonoids, tannins, alkaloids, terpenoids, and phenolic compounds are examples of plant-derived bioactive substances with antioxidant, anti-inflammatory, antibacterial, and antiglycation qualities. These phytochemicals play a critical role in reducing the production of AGEs by inhibiting oxidative damage and neutralizing free radicals.

DOI: 10.48175/IJARSCT-35831

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Eucalyptus globulus, sometimes referred to as Tasmanian blue gum or blue gum, is a member of the Myrtaceae family and is well known for its industrial and medicinal use. The plant is widely grown across many tropical and subtropical parts of the world, but it is native to Australia and Tasmania. The therapeutic benefit of eucalyptus leaves is attributed to their abundance of essential oils, particularly eucalyptol (1,8-cineole), as well as a variety of phytoconstituents, including flavonoids, tannins, terpenoids, alkaloids, and phenolic compounds. The herb has long been used to treat fever, wounds, inflammation, microbial infections, and respiratory conditions.

II. MATERIAL AND METHODS:

Plant Profile: Eucalyptus globulus

Fig.no.1 Eucalyptus globulus leaves

Common Name: Blue Gum, Tasmanian Blue Gum
Botanical Name: Eucalyptus globulus
Family: Myrtaceae
Description:The tall evergreen Eucalyptus globulus, sometimes called Blue Gum, is a member of the Myrtaceae family of trees. Originally from Australia and Tasmania, it has extensive industrial and therapeutic value, making it a popular choice for cultivation in temperate, tropical, and subtropical climates. The tree has a straight trunk covered with smooth, bluish-grey bark that peels off in strips and can reach heights of 30 to 70 meters.
The presence of essential oils gives the leaves their fragrant quality. Mature leaves are long, thin, lance-shaped, and dark green, but juvenile leaves are opposite, wide, and bluish-green. The creamy white, nectar-rich blooms are solitary. Often referred to as a "gumnut," the fruit is a woody capsule that contains many tiny seeds.
The plant is frequently planted beside highways, on plantations, and in forested regions since it grows well in sunny, well-drained soils. It is prized for its lumber, essential oil, and therapeutic qualities
Chemical Constituents:
• Essential oils (mainly eucalyptol/1,8-cineole) – major active constituent responsible for medicinal properties
• Flavonoids – antioxidant compounds
• Tannins – possess antimicrobial and astringent properties
• Phenolic compounds – contribute to antioxidant activity

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• Terpenoids and monoterpenes – responsible for anti-inflammatory effects
• Alkaloids – present in small quantities
• Resins and waxes – present in leaves and bark
• Organic acids and carbohydrates – primary metabolites

Uses:

1. Medicinal Uses

• Antiglycation activity: aids in preventing the production of AGEs, or advanced glycation end products.
• Antioxidant: flavonoids and phenolic substances scavenge free radicals.
• Anti-inflammatory: lessens inflammation in tests
• Antimicrobial: efficient against certain fungi and bacteria
• Expectorant and decongestant: often used for respiratory conditions, colds, and coughs
• Eucalyptus oil is an antiseptic used in preparations for disinfectants and wound cleansing.

2. Applications in Industry

• Production of essential oils: Eucalyptus oil is mostly derived from leaves.
• The timber and paper business uses wood for building, pulp, and paper.
• Fuelwood: Due to its quick growth, it is frequently utilized as firewood.
• Products that repel insects, such as oil used in sprays and repellents for mosquitoes

3. Customary and Folk Applications

• Inhaling steam from leaves to relieve congestion in the nose and cough
• External use of leaf paste and oil for mild injuries and sore muscles
• Used in conventional treatments for respiratory infections and fever

4. Decorative Use

• Because of its towering stature and fragrant leaf, it is grown as a shade and decorative tree.

III. METHODOLOGY

EXTRACTION OF PLANT EXTRACT:

Step 1: Weighing

Accurately weigh 50 g of powdered leaf sample using an electronic weighing balance.

Step 2: Filling the Thimble

Transfer the powder into a cellulose extraction thimble.

The thimble prevents solid particles from mixing with solvent.

Step 3: Assembly of Soxhlet Apparatus

The Soxhlet apparatus consists of:

Round-bottom flask

Extraction chamber

Condenser

Heating mantle

Step 4: Addition of Solvent

Add 300–500 mL methanol or ethanol into the round-bottom flask.

Why methanol/ethanol?

These solvents effectively extract:

Phenols

Flavonoids

Alkaloids

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Glycosides
Step 5: Heating
Heat the solvent at 60–70°C.
The solvent evaporates and moves upward into the condenser.
Step 6: Condensation
Cold water circulating through the condenser cools the solvent vapor.
The vapor condenses into liquid form and drips onto the plant powder.
Step 7: Extraction Cycle
The chamber fills with solvent.
When the solvent reaches siphon level, it automatically drains back into the flask carrying dissolved phytochemicals.
This cycle repeats continuously for 6–8 hours.
Usually 8–12 cycles are sufficient.
Step 8: Completion
Extraction is complete when the solvent in siphon tube becomes colorless.
Step 9: Filtration
The extract is filtered using Whatman filter paper No.1.
Purpose: To remove suspended particles.
Step 10:Concentration of Extract
The filtrate contains solvent + extracted phytochemicals.
It is concentrated by:
Water Bath Method
Heat at 40–50°C until solvent evaporates.
OR
Rotary Evaporator
Used under reduced pressure for efficient solvent removal.
The concentrated crude extract is:
Sticky/semi-solid
Dark green/brown
The extract is weighed and percentage yield is calculated:
Step 11:. Storage of Extract
The crude extract is transferred into sterile airtight containers.
Stored at 4°C in refrigerator.
Purpose: Prevents:
Oxidation
Microbial growth
Chemical degradation

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Fig.no. 2 Soxhlet Apparatus

Preliminary Phytochemical Screening:

A. Alkaloid test:

TEST	OBSERVATION
Mayer's Test Take 2-3 ml of plant extract in a test tube. Add few drops of dil.HCL and warm gently. Add Mayer's reagent	Pale yellow precipitate
Dragendorff's Test Take 2-3 ml of plant extract in a test tube. Add few drops of dil.HCL and warm gently. Add Dragendorff's reagent	Reddish-brown precipitate
Wagner's Test Take 2-3 ml of plant extract in a test tube. Add few drops of dil.HCL and warm gently. Add Wagner's reagent	Reddish-brown precipitate

Table No.1 Alkaloid test

B. Flavonoids Test:

Shinoda Test Take 2-3 ml of plant extract in a test tube.	Pink colour are observed

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Add small Zinc Dust Add few drops of dil.HCL	

Table No.2 Flavonoids Test

C. Saponin Test:

Foam Test Take 1ml plant extract. Add 5-10 ml distilled water. Shake vigorously for 30 sec. Let it stand for 10-15 min.	Persistent foam (>1cmheight)

Table No.3 Saponin Test

D. Tannins Test:

Take 2ml plant extract. Add 2-3 drops of $Fecl_3$ solution.	Black color observed

Table No.4 Tannin Test

E. Terpenoids Test

Salkowski Test Take 2ml plant extract. Add 2ml chloroform. Carefully add 2-3 ml conc. H_2SO_4 along the side of the test tube.	Brown ring at junction

Table No.5 Terpenoids Test:

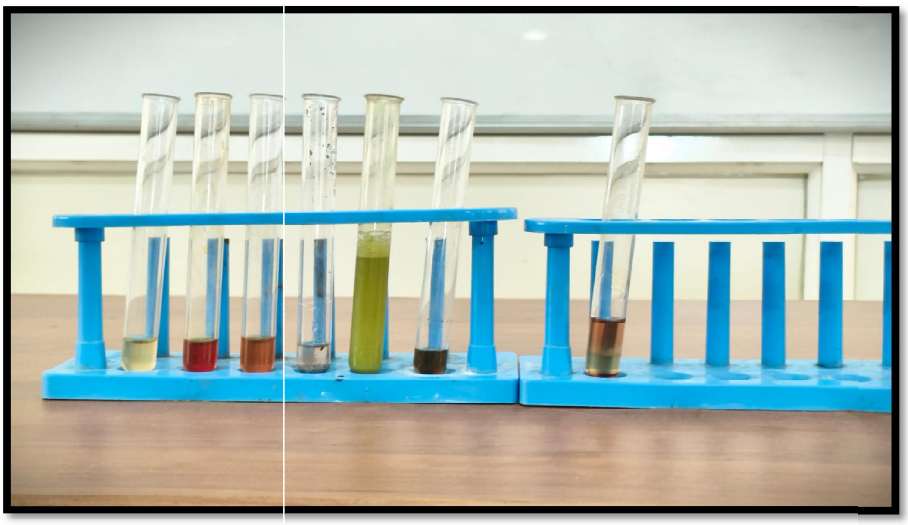

Fig.no.3 Preliminary Phytochemical Screening

ASH VALUE:

The ash value of Eucalyptus globulus is determined by the incineration (heating) method used in pharmacognosy to measure the inorganic residue left after burning the plant material.

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Principle:
When the powdered drug is heated at high temperature, all organic matter burns off and the remaining residue is called ash. It indicates the presence of:
• Natural mineral content
• Adhering dirt, sand, or impurities
• Adulteration
• Heating / Incineration Method for Ash Value Determination
Procedure:
1. Weigh about 2–3 g of air-dried powdered Eucalyptus globulus.
2. Place it in a previously ignited and weighed silica crucible.
3. Heat gently at first to avoid loss due to smoke.
4. Then incinerate in a muffle furnace at about 450–600°C until the sample becomes white, indicating absence of carbon.
5. Cool in a desiccator and weigh the ash.
Formula:

$$\text{Ash Value (\%w/w)} = \frac{Weight\ of\ Ash}{Weight\ of\ air\ dried\ crude\ drug} \times 100$$

LOSS OF DRYING
Principle:
The powdered drug is heated at 105°C until constant weight is obtained. The weight loss indicates moisture and volatile components removed during drying.
Procedure:
1. Weigh about 2–5 g of powdered drug.
2. Place in a previously dried and weighed evaporating dish.
3. Dry in hot air oven at 105°C.
4. Cool in desiccator and weigh.
5. Repeat until constant weight is obtained.
Formula:

$$\text{Loss of Drying (\%w / w)} = \frac{Initial\ weight - Finial\ weight}{Initial\ weight} \times 100$$

PHARMACOLOGICAL ACTIVITY
Anti-Glycation Assay By Titration Method
Principle-
Glycation reaction leaves free reducing sugars in solution. These are estimated by titration. The lower the titration volume, the higher the anti-glycation activity.
Key Idea-Instead of control flask:
Use 0 µg/mL (blank mixture) as reference titre value
Then compare all test samples against it.
Materials-
Fructose (1% or 0.5%), Gelatin , Plant extract (20–100 µg/mL),Fehling's solution A & B, Methylene blue indicator, Distilled water
Procedure-
1. Prepare Reaction Mixture
For each tube:
2 mL fructose solution

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2 mL Gelatin solution
1 mL extract (or water for blank)

2. Set of Tubes
You prepare:

TUBE	EXTRACT
Blank(V_0)	o µg/Ml
T1	20 µg/mL
T2	40 µg/mL
T3	60 µg/mL
T4	80 µg/mL
T5	100µg/mL

Incubation-
60°C water bath for1 hour.
Titration-
For each tube:
1. Take Fehling's A + B (5 mL each)
2. Boil gently
3. Titrate with incubated sample
4. Add me.thylene blue near endpoint
5. Record volume

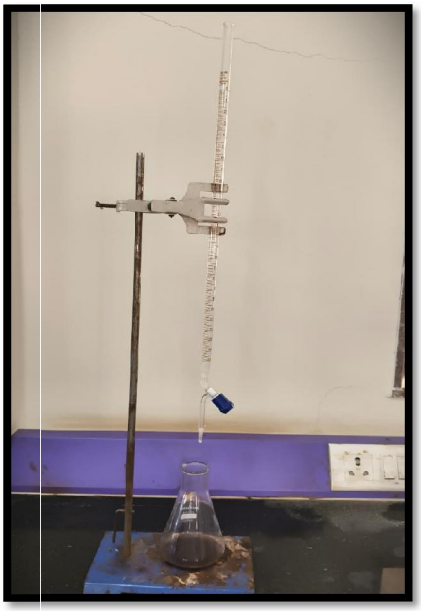

Fig.no.4 Anti-Glycation Assay By Titration Method

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IV. RESULTS AND DISCUSSION:

PRELIMINARY TESTS:

Alkaloid test:

TEST	OBSERVATION	INFERENCE
Mayer's Test Take 2-3 ml of plant extract in a test tube. Add few drops of dil.HCL and warm gently. Add Mayer's reagent	Pale yellow precipitate	Alkaloid is present.
Dragendorff's Test Take 2-3 ml of plant extract in a test tube. Add few drops of dil.HCL and warm gently. Add Dragendorff's reagent	Reddish-brown precipitate	Alkaloid is present.
Wagner's Test Take 2-3 ml of plant extract in a test tube. Add few drops of dil.HCL and warm gently. Add Wagner's reagent	Reddish-brown precipitate	Alkaloid is present.

Table No.6 Result of Alkaloid test

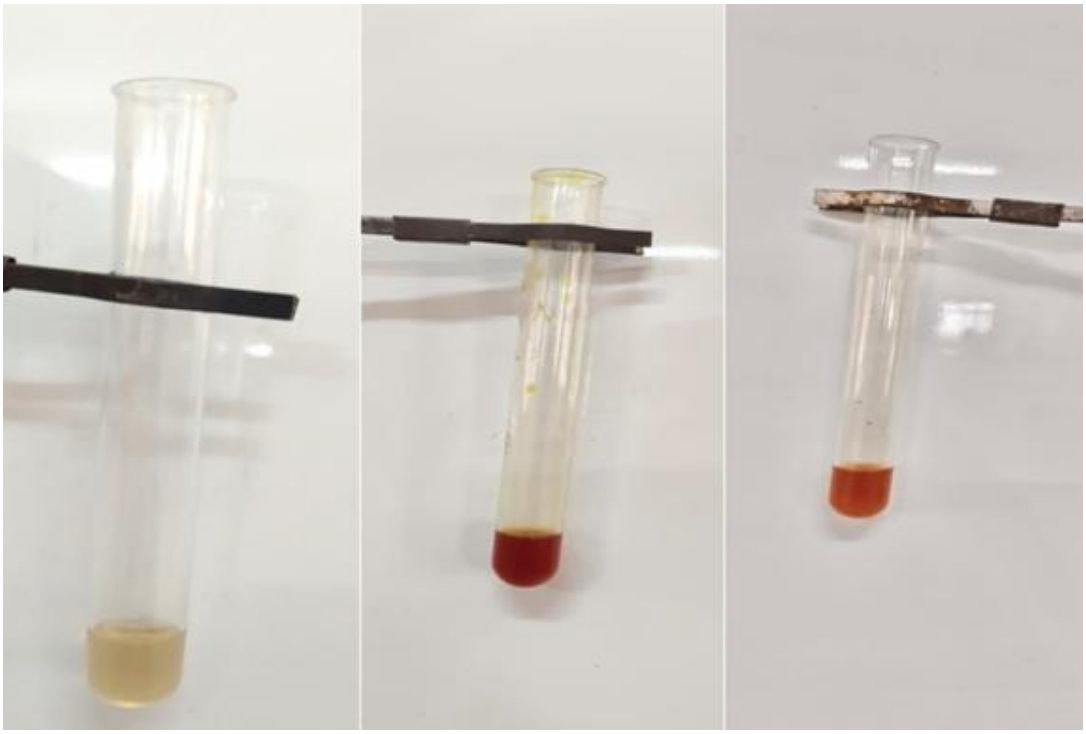

Fig.no.5 Result of Alkaloid test

Flavonoids Test

Shinoda Test Pink colour are observed Falvonoid is present

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Table.No.7 Result of Shinoda test

Shinoda Test	Pink colour are observed	Falvonoid is present

Fig.no.6 Result of Shinoda test

Table.No.8 Result of Saponin test

Foam Test	Persistent foam (>1cmheight)	Saponin is Present.

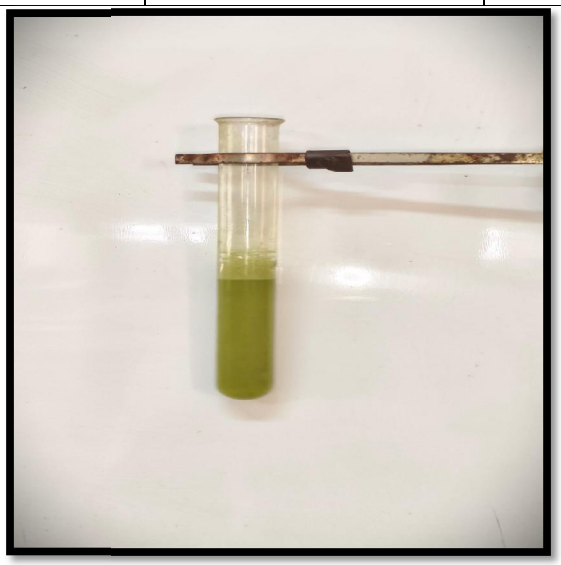

Fig.no.7 Result of Saponin test

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Tannins Test

Tannis Test	Black color observed	Tannin is Present

Table.No.9 Result of Tannin test

Fig.no.8 Result of Tannin test

Terpenoids Test

Salkowski Test	Brown ring present at junction of two chemicals.	Terpenoids is present

Table.No.10 Result of Terpenoids test

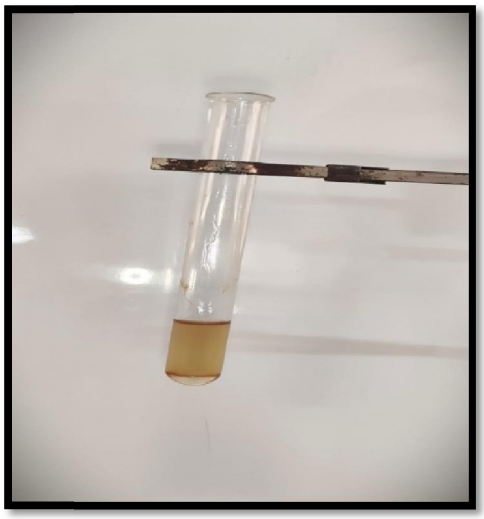

Fig.no.9 Result of Terpenoids test

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ASH VALUE

Ash Value (% w/w) = 5%

Fig.no.10 Ash Value

2.3 LOSS OF DRYING

Loss on Drying (% w/w) =10%

Fig.no.11 Loss of Drying

2.4 Anti-Glycation Assay By Titration Method:

Observation Table:

Concentration (µg/mL)	Initial reading	Final reading	Titre value	Percentage Inhibition
0 (Blank)	0.0	10.0	10.0	-
20	0.0	9.5	9.5	5 %
40	0.0	8.7	8.7	13%
60	0.0	6.5	6.5	35%
80	0.0	6	6	40%
100	0.0	5	5	50%

Table No.11 Observation Table of Anti-Glycation Assay

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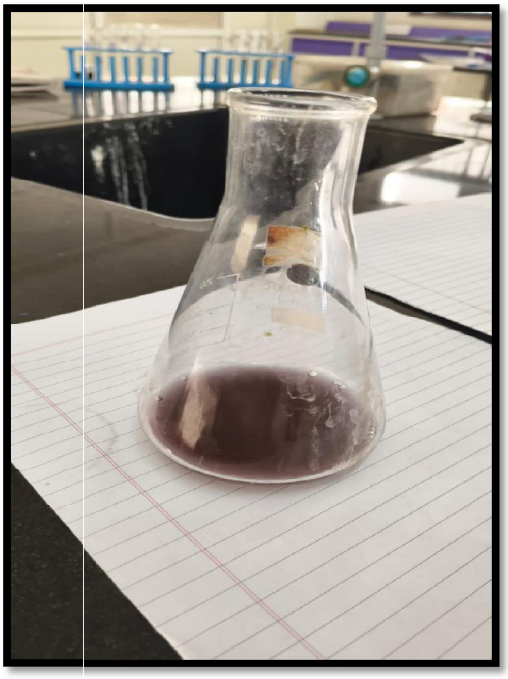

Fig.no.12 Result of Anti-Glycation Assay By Titration Method

VI. CONCLUSION

In vitro antiglycation tests, physicochemical analysis, and phytochemical screening were used in this work to assess the antiglycation activity of Eucalyptus globulus leaf extract. The findings showed that because Eucalyptus globulus contains a variety of bioactive phytoconstituents, it has considerable therapeutic potential. Important secondary metabolites with antioxidant, anti-inflammatory, and medicinal qualities, including alkaloids, flavonoids, tannins, saponins, and terpenoids, were found by preliminary phytochemical screening. Advanced glycation end products (AGEs), which are linked to diabetes, aging, and a number of chronic illnesses, may be inhibited by these substances.

The crude drug's acceptable purity and moisture content were confirmed by the physicochemical criteria, such as ash value (5%) and loss on drying (10%), which validated its suitability for experimental usage. Phytochemicals were effectively recovered from the leaves using the Soxhlet extraction technique with methanol or ethanol, yielding a concentrated crude extract appropriate for further study.

The titration approach of the antiglycation test demonstrated concentration-dependent glycation inhibition. Strong antiglycation action was demonstrated by the percentage inhibition rising from 5% to 50% as the extract concentration rose from 20 µg/mL to 100 µg/mL. Eucalyptus globulus may successfully lower glycation and AGE production, according to the maximal inhibition at 100 µg/mL.

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