



Research Article

In- Vitro Anti-inflammatory Activity Of Glochidion Zeylanicum A. Juss. Leaves Extract And It's Characterization

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ARTICLE INFO

Received: 13 Jan 2024

Accepted: 17 Jan 2024

Published: 30 Jan 2024

Keywords:

Alzheimer's disease (AD),
Acetylcholinesterase (AChE),
Eburnane Alkaloids, Lead
Molecules

DOI:

10.5281/zenodo.10569171

ABSTRACT

The goal was to create in-vitro anti-inflammatory activity of glochidion zeylanicum. Leaves extract and its characterization. The drug was evaluated by using different in-vitro anti-inflammatory activity. GC-MS, UV, and FT-IR were used for the characterization of hydromethanolic leaf extract. According to the findings, Glochidion Zeylanicum A. Juss by preventing the hypnotically induced hemolysis of HRBCs in a dose-dependent manner (25, 50, 75, 100 µg/mL), hydromethanolic extract was able to provide membrane stabilization. The Glochidion Zeylanicum A. Juss. extract was also capable of inhibiting HRBCs at 58.42% which was comparable to that of diclofenac sodium at 74.73%. When compared with hydromethanolic extract methanolic extract showed a good anti-inflammatory effect. From the current research, we can conclude that glochidion zeylanicum A. Juss. Possesses an appreciable anti-inflammatory effect against human red blood membrane stabilization assay. Further studies are required to determine the possible mechanism behind its anti-inflammatory action.

INTRODUCTION

Inflammation is a host defense mechanism to eliminate pathogens and to initiate the healing process, but the uncontrolled or overproduction of inflammatory products can lead to injury of host cells, chronic inflammation and also chronic diseases. Beginning with an inflammation, the cells undergo activation and release inflammation

mediators which can either be cell-derived or plasma-derived. Cell-derived mediators include vasoactive amines, cytokines, nitric oxide, prostaglandins, thromboxane A₂ (TX A₂), prostacyclin, leukotrienes and platelet-activating factor. Chronic inflammation is associated with the infiltration of mononuclear immune cells, macrophages, monocytes neutrophils, fibroblast

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



activation, proliferation (angiogenesis) and fibroblasts. Inflammation may be potentially harmful, causing life-threatening, hypersensitivity reactions and progressive organ damage. Platelets also play roles in inflammation where they promote vascular permeability and recruit inflammatory cells. They contain alpha, dense and lysosomal granules which store many important inflammatory and immune mediators that are rapidly released after platelet activation. Anti-inflammatory medications are administered to lessen the harm brought on by inflammation or oxygen-reactive species. Inflammation is treated with steroids and NSAIDs (non-steroidal anti-inflammatory medicines). Which possess many adverse side effects especially gastric irritation leading the development of gastric ulcers. Non-steroidal Anti-inflammatory Drugs (NSAIDs) represent a common class of anti-inflammatory and analgesic drugs for alleviating symptoms associated with inflammation by inhibiting COX. The cyclooxygenase enzyme occurs in two isoforms, COX-1 and COX-2. The World Health Organisation (WHO) estimates that around three-quarters of the world's population relies on herbal remedies for their health. The most widely accepted traditional systems with extensive study in pharmacognocny, chemistry, pharmacology, and clinical treatments are Ayurveda and Chinese medicine.

PLANT PROFILE:

The genus *Glochidion* commonly called as cheese trees or button wood trees consisting of 300 species. Several triterpenoids, triterpenoid glycosides and alkaloids are known to be constituents of the plants belonging to the genus *Glochidion*. According to biological studies on *Phyllanthus* species, several of the genus's members have properties that prevent the growth of tumours and have action of the antiviral hepatitis B virus, anti-angiogenic, lipid-lowering, anti-diabetic, antiherpetic, anti-HIV, and

antiplasmodial properties. However, no biological studies of *G. zeylanicum* A. Juss have been found in literature to date. Hence, in the present study,



Figure 1: *Glochidion zeylanicum* A. Juss.

Synonym:

Phyllanthus zeylanicus (Gaertn.) Mull.Arg., nom. illeg.

Family: Phyllanthaceae

Vernacular Names: Neeru kukke (Kannada); **Kumbal (Tamil);** Itepulla, Pageri, Itepulla (Telugu); kalchia, berlu (Oria); Neevetti, Pannimutti (Malayalam).

Other common names: Ghoda/ Askand/ Ashwagandha

Hang Kong Abacus Plant

Kokamani moram- Tamil

Habitat:

Plains also have woods that are evergreen and semi-evergreen.

Uses:

System medicines used in Folk medicine, Siddha

Collection Of Plant Material:

The plant *G. Zeylanicum* (Gaertn) A. Juss was collected from the Sahyadri forest. It was authenticated by the Department of Botany, Yashwantrao Chavan College of Science, Karad. A herbarium was prepared and deposited in the Dept. of botany, for further reference. The plant was identified as *G. Zeylanicum* (Gaertn) A. Juss (Phyllanthaceae). and was certified under Voucher No: EVU 001.

Experimental Method:

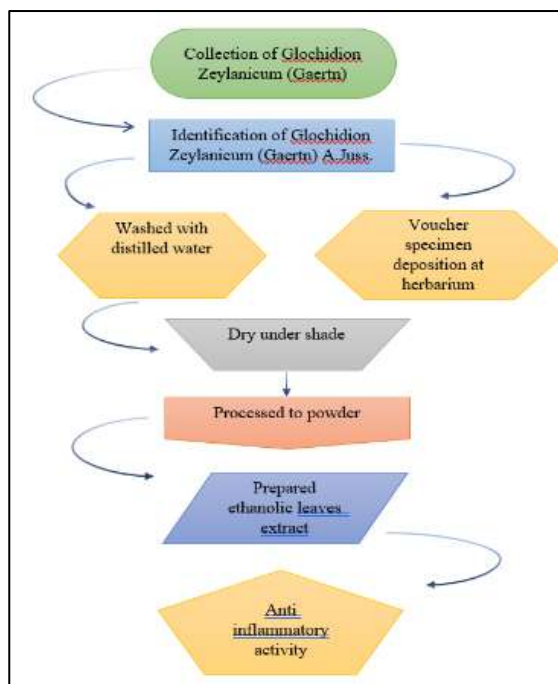


Preparation of successive extraction method:

To separate and clean the leaves, water was used. The dried leaves of the *G. Zeylanicum* (Gaertn) A. Juss. with ethanol using a Soxhlet apparatus, performing roughly 10 cycles each batch for one batch. It was necessary to keep extracting until the solvent in the thimble was crystal clear. A Whatman filter no. 42 was used to filter the extract after distillation to recover the solvent.



G. Zeylanicum plant were ground into a coarse powder (40-size mesh), and then 150 g of the powder was treated to a series of hot continuous extractions. Concentrated extracts were used to fire the mind. A solid mass was eventually produced, and it was stored in a glass container in the fridge. To study phytochemistry and conduct pharmacological experiments, dried concentrated extracts were used.



Phytochemical Screening:

The outcomes of extracted contents of the whole plant (in different solvents) tested for the presence or absence of various phytochemicals (in qualitative form) are noted in Table 1. The results show that *G. Zeylanicum* (Gaertn) A. Juss. the plant contains a maximum of ten types of phytochemical groups, such as – alkaloids, flavonoids, proteins, carbohydrates, steroids, glycosides, Phenols, saponins, and terpenoids.

GC-MS Analysis:

Preparation of extract:

Leaves powder of *G. Zeylanicum* (Gaertn) A. Juss. were shade dried 1gm of the powdered Eppendorf tube were soaked in 95% ethanol for 12 h. To eliminate the sediments and residues of water from

the filtrate, the extract was then filtered through Whatman filter paper No. 42 with 2gm sodium sulfate. Sodium sulfate and filter paper were moistened with 95% ethanol before filtering. After that, nitrogen gas was blasted into the solution to concentrate the filtrate. The extracted plant material included both polar and non-polar photo components.

GC Condition and Identification of Compounds:

GC-MS analysis of Ethanolic leaves extract of *G. Zeylanicum* (Gaertn) A. Juss. was carried out on instrument GCMS-TQ8050 Ultra, equipped with a Vf-5ms fused silica capillary column of (30m×0.25mm×0.25µm). 1 µl of respective sample manually injected in splitless mode. The

column head pressure was programmed to 54.4kPa. Column temperature maintained at 50,180 and 250°C with a hold time of 2.00, 2.00, and 2.00 min. respectively. The GC-MS interface was programmed at 2700C. In the fuel scan range, 45-700(m/z) were recorded. The start-end time was 3.00-46.00 min. The identifying information for the compound was done by comparing mass spectra Shivaji University, Kolhapur (CFC) using a GC-MS model; Shimadzu GC-2010 Plus, Tokyo, Japan.

Fourier Transform Infrared Spectrophotometer (FTIR) Analysis:

Fourier transform infrared spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. Dried powders of solvent extract were utilized for FTIR analysis of plant material. The powdered sample of ethanolic leaves extract of *G. Zeylanicum* (Gaertn) A. Juss. was loaded FTIR spectroscope (JASKO, FTIR-4600) with a scan range 4000-650cm⁻¹ with a resolution of 4cm⁻¹.

UV Spectroscopy:

UV spectroscopy is a form of absorption spectroscopy in which molecules absorb light in the ultra-violet range (200–400 nm). The electrons are excited from their ground state to a higher energy state as a result of the ultraviolet radiation being absorbed. The difference between the energy of the ground state and higher energy levels and the energy of the absorbed ultraviolet light.

The functional groups and metabolites present in the leaves extract of *G. zeylanicum* (Gaertn) A. Juss., the extracts were examined under visible and UV light for proximate analysis. For UV-Vis spectrophotometer analysis, the extract were prepared to contain 10mg of leaf extract in 100mL of the volumetric flask, the volume adjusted up to 100mL with the ethanol, respectively to get a final concentration of 100µg ml⁻¹ for GZ leaf extract.

Suitable aliquots of 100 µg ml⁻¹ solutions were diluted up to the mark with ethanol to get the concentration range 1,2,3,4 and 5 µg ml⁻¹ for GZ leaf extract the absorbance was recorded for leaves extract respectively by using UV-visible spectroscopy (PC Based Double Beam Spectrophotometer 2202)

IN-VITRO ANTI-INFLAMMATORY ACTIVITY:

Preparation of Erythrocyte Suspension:

According to the procedure given by Shin de et al., erythrocyte suspension was created. A healthy human subject's whole blood was taken. The blood was centrifuged at 3000 rpm for 5 minutes in heparinized centrifuge tubes before being rinsed three times with normal saline (0.9% NaCl) of an equivalent amount. The blood volume was determined following centrifugation, and it was then reconstituted as a 10% (v/v) suspension in an isotonic buffer solution (10 mM sodium phosphate buffer pH 7.4). The buffer solution was composed chemically of NaH₂PO₄ (g/L) (0.2), Na₂HPO₄ (1.15), and NaCl (9.0).

Heat-Induced Hemolysis:

Briefly, 2.95 mL of phosphate buffer (pH 7.4) was combined with 0.05 mL of blood cell suspension and 0.05 mL of hydro methanolic extracts (25, 50, 75, and 100 µg/mL) of leaves. In a water bath that was shaking, the mixture was incubated at 54 o C for 20 min. After the combination had been incubated, it was centrifuged for three minutes at a speed of 2500 rpm to measure the supernatant's absorbance at 540 nm. Phosphate buffer solution served as the experiment's control and was measured using a VIS spectrometer (Equiptronics, Mumbai). Using the following equation based on Okoli et al.'s research, the degree of hemolysis was determined.

% inhibition of hemolysis = 100 x (1 - A₂/A₁)
where A₁ = absorption of the control, and A₂ = absorption of the test sample mixture.

RESULT AND DISCUSSION**Table 1: Phytochemical constituents present in Ethanolic extract of *Glochidion Zeylanicum* (Gaertn) A. Juss.**

Sr. No	Phytochemicals	<i>Glochidion Zeylanicum</i> (Gaertn) A. Juss. Ethanol Extract
1.	Alkaloids	+
2.	Steroids	-
3.	Flavonoids	+
4.	Glycosides	-
5.	Tannins	+
6.	Saponins	+

Table 2: Phytochemical identified in the ethanolic extract of the *G. Zeylanicum* (Gaertn) A. Juss. By GC-MS

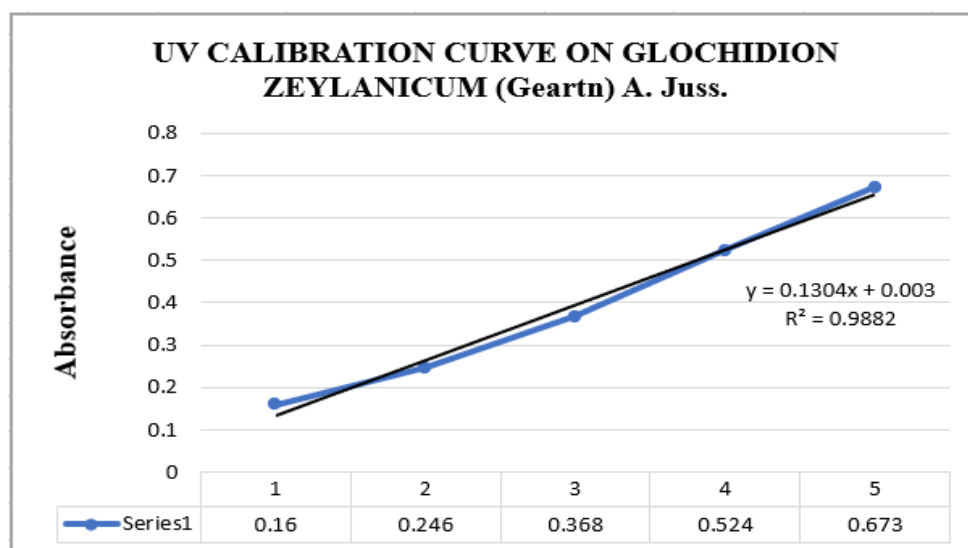
Sr. No	RT	Name of the compound	Molecular formula	MW	Peak area (%)
1.	31.349	Tetradecanoic acid, ethyl ester	C ₁₆ H ₃₂ O ₂	256	0.67 1.46
2.	33.577	Neophytadiene	C ₁₇ H ₃₄ O ₂	278	34.33
3.	36.334	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	0.69
4.	38.536	Ethyl 15 methyl hexadecanoic	C ₁₉ H ₃₈ O ₂	298	18.00
5.	38.921	Phytol	C ₂₀ H ₄₀ O	296	10.54
6.	39.908	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308	19.61
7.	40.037	9,12,15 Octadecatrienoic acid, ethyl ester,	C ₂₀ H ₃₄ O ₂	306	8.47
8.	40.574	Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	312	0.73
9.	44.303	Methyl 19-methyl-eicosanoid	C ₂₂ H ₄₄ O ₂	340	0.62
10.	45.410	Trichothec-9-en-8-one, 12,13-epoxy-4-hydroxy-	C ₁₉ H ₂₄ O ₅	332	0.67 1.46

Ethanolic extract of the substance in *G. zeylanicum* (Gaertn) A. Juss. was identified by GC-MS analysis (Figure 1). The active compounds along with their chemical formula, molecular weight (MW), retention time (RT), and concentration (%) are presented in Table 2 Ten components present in the *G. zeylanicum* (Gaertn) A. Juss. were Hexadecanoic acid, ethyl ester

(34.33), 9,12,15 Octadecatrienoic acid, ethyl ester (19.61%), Phytol (18.00%) Octadecanoic acid, ethyl ester (8.47%), Neophytadiene (1.46%), Methyl 19-methyl-eicosanoate (0.73%) and various other compounds were identified as low level.

Table 3: UV- Vis. spectrum peak values of G. zeylanicum (Gaertn) A. Juss. ethanol extract

Sr. No	Wavelength (nm)	Absorption Peak
1	202.4	0.160
2	202.4	0.246
3	202.4	0.368
4	202.4	0.524
5	212.0	0.673

**Figure 3: UV-Vis Spectral analysis of G. zeylanicum (Gaertn) A. Juss. ethanol extract****In-Vitro Anti-inflammatory activity:**

To screen in vitro anti-inflammatory activity of G. zeylanicum (Gaertn) A. Juss extract heat-induced membrane stabilization method was carried out. The extract at different concentrations was incubated with phosphate buffer (pH 7.4) in controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. The results are summarized in (Figure 4, and Table 4), which show that extract G. zeylanicum (Gaertn) A. Juss showed promising in vitro anti-inflammatory activity in a concentration-dependent manner. Using acetylsalicylic acid as a standard drug and

compared with ethanolic extract to determine anti-inflammatory activity. The heat-induced anti-inflammatory test revealed that crude ethanolic extract of G. zeylanicum (Gaertn) A. Juss. (75 μ g/ml) and positive control ASA (75 μ g/ml) have 75.78% and 60.52% inhibition of red blood cell (RBC) hemolysis

Table 4: Result of Invitro Anti-inflammatory Study

Sr. No	Concentration (μ g/ml)	Percentage Inhibiton	
		Standard	Extract
1.	20	73.68%	55.78%
2.	50	74.73%	57.89%
3.	75	75.78%	60.52%

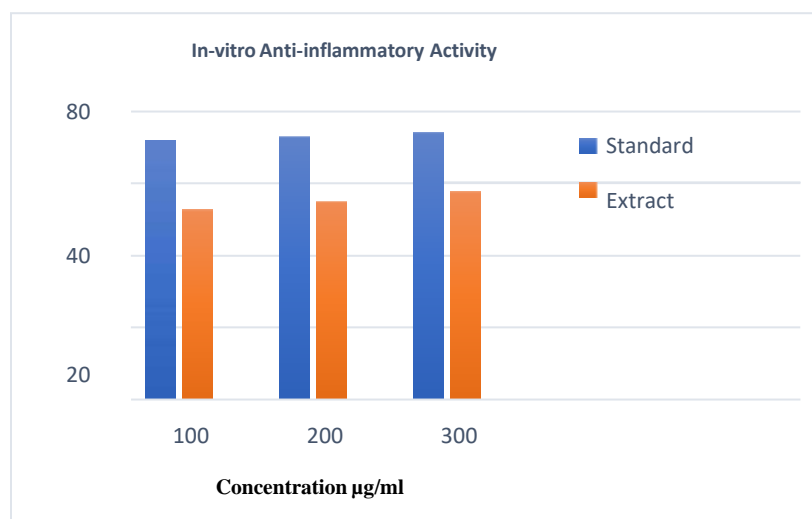


Figure 4: Graphical representation of % inhibition of extract compared with the standard drug against Heat-Induced Membrane Stabilization

CONCLUSION

As per the study carried out entitled In-vitro anti-inflammatory activity of *Glochidion zeylanicum* A. Juss. Leaves extract and its characterization.” We concluded that, The extract was successfully obtained by continuous heat extraction method and confirmed by possible evaluation of crude extract. Further, the various characterization GC-MS, FT-IR, and UV by results were carried out. In-vitro anti-inflammatory activity was carried out by using Membrane Lysis Assay Sample *Glochidion Zeylenicum* A. Juss. Hydromethanolic extract showed good activity when compared with the standard drug diclofenac sodium.

ACKNOWLEDGEMENT:

The authors express their thanks to Dr. Girish G. Potdar Yashwantrao Chavan College of Science, Karad for authentication of the plant material. The authors are thankful to Dr. Archana S. Murgunde Assistant Professor (RCPK), Dr. Vijay Salunkhe Professor (RCPK) Dr. Shrinivas K. Mohite. Principle(RCPK) Mr. Siddheshwar Mule and management of Rajarambapu College of Pharmacy, Kasegaon, for providing the facilities necessary to carry out this research work.

CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

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HOW TO CITE: Ek Nath V. Unde, Snehal B. Fand, Amol S. Darade, Ganesh R. Gosavi, In- Vitro Anti-inflammatory Activity Of Glochidion Zeylanicum A. Juss. Leaves Extract And It’s Characterization, *Int. J. of Pharm. Sci.*, 2024, Vol 2, Issue 1, 831-840. <https://doi.org/10.5281/zenodo.10569171>

