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# "Siddha Science: Unlocking the Herbal LCZ Mixture's Anti-Inflammatory Secrets for Modern Healing"

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**Abstract**

*Introduction: Leucas aspera, Coccinia grandis, and Zingiber officinale (*LCZ*)* are commonly used in Asia's Ayurvedic, Siddha, and Unani conventional medical practices. Different portions of these plant materials have historically been employed in traditional medicine for various functions, like anti-diabetic, anti-bacterial, anti-inflammatory, etc. Siddha-based formulation mixtures for medicinal uses are considered an efficient alternative to synthetic substances because of their side effects. **Aim and Objective:** The research aims to find an *in-vitro* anti-inflammatory action of the herbal-based Siddha formulation mixture of *Coccinia grandis, Leucas aspera, and Zingiber officiale.* **Methods:** The anti-inflammatory activity of the herbal-based Siddha formulation mixture of *Coccinia grandis, Leucas aspera, and Zingiber*

*officinale* was evaluated using in vitro-based assays: protease inhibition assay, protein denaturation inhibition assay, and phytochemical analysis. This study mainly focuses on finding the most potent plant extracts and their effectiveness. All data are presented as the mean ± standard deviation for all in vitro assays tested, and each analysis was done in triplicate. One-way analysis of variance (ANOVA) was performed using MINITAB 15 software and Pearson's correlation coefficient (r) with the level of significance ($p \leq 0.05$) (2-tailed) **Results:** The LCZ sample is analyzed for the protease inhibition assay, and the higher (500µg) concentration of the specimen mean value was identified as 37.4864%. In the albumin denaturation assay, the higher (500µg) concentration of the specimen mean value was 80.9151%. The LCZ sample's IC50 value was 68.56 µg/ml for the protease inhibitory assay. For the primary albumin denaturation assay, it was 42.66 µg/ml. **Conclusion:** The outcomes of our study propose that all portions of plants exhibit potent anti-inflammatory action. The existence of most general phytochemicals in LCZ extract may account for their medicinal impacts.

**Keywords:** *Coccinia grandis, Leucas aspera, Zingiber officinale,* Albumin Denaturation, Protease Inhibition, Siddha medicine

**Introduction**

The disruption of tissue homeostasis brought about the existence of biological and physicochemical substances in the body, resulting in inflammation, a natural defense mechanism [1]. The body starts the process of inflammation as a defense against localized tissue injury. It is a sophisticated interaction between connective tissue, skeletal elements, and blood vessels. The word "inflammatory reaction" describes the physiological processes in the tissue due to an invasive pathogen or a toxic substance. In the present study, ethanol extracts of the aerial parts of *Leucas aspera*, *Coccinia grandis*, and the rhizome of *Zingiber officinale* are employed to make herbal products.

*Leucas aspera* is a medicinal plant from the *Lamiaceae* family, *Leucas* genus. It is also called *Thumbai* in Tamil or *Dronapushpi i*n Sanskrit and is found all over India, from the Himalayan Mountains to Ceylon. The plant has historically been employed as a pesticide and antipyretic. Flowers are highly esteemed as a stimulant, aperient, diaphoretic, insecticide, and emmenagogue. Leaves are beneficial in persistent rheumatism, psoriasis, and other skin eruptions. Snake wounds are treated with bruised leaves. Numerous phytochemicals, such as triterpenoids, sitosterols, nicotine, glycoside, diterpenes, isoamyl propionate, ceryl alcohol, phenolic compounds, and alkaloids, are present and play a significant role in their use as anti-inflammatory, antioxidant, antifungal, antimicrobial, anti-diabetic, analgesic, and anti-asthmatic medications [2]. Previous studies such as Phytopharmacological studies, analgesic, antipyretic, anti-inflammatory, antioxidant, anti-diabetic, anti-cancer [3], pharmacological, preliminary phytochemical investigation, Allelopathic potentiality [4], cytotoxic activity, antimicrobial, antinociceptive, larvicidal activity, anti-venom activity, antifungal, hepatoprotective activity, antihyperglycemic activity, anti-asthmatic activity conducted in *Leucas aspera* [5].

*Coccinia grandis, from the Curcurbitaceae* family, *Coccinia* Genus, is a climber plant cultivated in India. Telachucha, Tindora, Scarlet-fruited gourd, and Ivy-gourd are some of its more well-known names. Oils and proteins from seeds may be helpful for industrial and dietary needs [6]. Fruits have been used in traditional medicine to cure jaundice, asthma, bronchitis, leprosy, and fever. It can also be used as an antiseptic and an expectorant. The plant's alcoholic liquid is used as an antioxidant and hypoglycemic medication. Polyprenol, extracted from ethanol extract, has anti-dyslipidemic properties [7]. It is also medicinally used for antipyretic, insecticide, antifungal, antimicrobial, antinociceptive, and cytotoxic activity. Critical raw materials for the manufacture of pharmaceuticals can be found in *Coccinia grandis*, including bioactive substances like secondary metabolites like alkaloids, glycosides, and saponins, as well as b-marine, lupeol, cucurbantioxidanthalandrol, cephalandrine, and flavonoids. The previous works like Pharmacological activities, pharmacognosy, antioxidant, anti-bacterial activity, hepatoprotective activity, anti-diabetic, antimicrobial, phytochemical and pharmacological screening, antiglycation property, antidyslipidemic activity, antihistaminic activity, anti-anaphylactic activity, antinociceptive activity, anthelmintic activity, antitussive activity, anti-inflammatory activity, anti-apoptotic activity, antiulcer activity, anti-collagenase, anti-elastase, anticancer conducted in *Coccinia grandis [8]*.

Ginger, the one anti-oxidant widely used spice throughout the globe roscoe, belongs to the Zingiberaceae group, Zingiber mill Genus, and is more commonly called "ginger." *ZingiberOfficinale (ginger)* rhizome is frequently used for medical purposes. The use of ginger in both communicable and non-communicable illnesses is emphasized in Siddha literature. Recent developments in analytical chemistry, cytology, and microbiology suggest using ginger to treat various disease conditions, in addition to suggestions made in Siddha and Ayurveda writings [9]. It is a perennial herbaceous plant material that cultivates to a height of about 1m [10]. Its leaves emerge from a branching rhizome. They are commonly used for headaches, indigestion, nausea, vomiting, cancer, autoimmune diseases, hypertension, hypercholesteremia, hyperuricemia, and bacterial infection. Previous works like pharmacological activity, anti-oxidant, antiobesity actions, antimicrobial investigation, anti-hypercholesterolaemic, and gastroprotective effects were conducted in *Zingiber officinale* [11]. The aim is to study the anti-inflammatory action in the case of albumin denaturation assay and protease inhibition assay. The novelty of our work is that herbal medicines are unprocessed or extracted products that have been separated from natural components. They are less toxic, less dangerous, and safer when compared to chemically produced medicines. An antioxidant action is made using the rhizome of *Zingiber Officinale, Coccinia Grandis,* and the aerial parts of the three chosen medicinal plants *LeucasAspera,* and *CocciniaGrandis.* A protease inhibition and albumin denaturation assay were employed to investigate the anti-inflammatory action.

**Materials and methods**

Phosphate buffer saline, sodium bicarbonate, HCL, Methanol, Trypsin, Casein, Perchloric acid and Test sample**,** and Acetylsalicylic acid chemicals were attained from Sigma Aldrich.

**Collection of Sample**

The test sample is collected in and around Trichy10.7672 ° N, 79.8449° E. The plant materials (Figure 1) were dried in the shade, made into powder using an electric mixer, and stowed in sterile, airtight containers.

**Preparation for Cold extraction (LCZ)**

The sample LCZ is measured at 10gm, mixed with 100ml of Methanol, and incubated at $4^0$C for 24 hrs. After incubation, the immersed material is separated by Muslin cloth and filtered using Whatman No.1 paper.

*(a) CocciniaGrandis* *(b) ZingiberOfficinale* *(c) LeucasAspera*

Figure 1. Plant extracts (a) *Coccinia Grandis, (b) Zingiber Officinale*and (c)*Leucas Aspera.*

**Qualitative study of phytochemicals**

**Carboxylic acid identification**

To the 1 ml of extract, 2 ml of sodium bicarbonate solution is introduced. The formation of color represents the existence of carboxylic acid.

**Tannin identification**

To the 2 ml of plant, 2 to 3 ml of 10 percent HCL is introduced and heated for 5 to 6 mins. The development of a reddish color signifies the existence of tannins.

**Steroids identification**

To the 0.5 ml extract, 5 ml of chloroform solution is introduced, and an equivalent quantity of concentrated $H_2SO_4$ is introduced. On the top layer, the development of a reddish color, and in the bottom layer, yellowish green colors showed the existence of steroids.

**Flavonoids**

To the 0.5 ml extract, 4 ml of 1 percent$NH_3$ismixed and add 1ml of concentrated sulphuric acid. The development of yellowish color designates the existence of flavonoids.

**Glycosides test**

**Born- Trager's**

The existence of glycosides is determined by adding 10% ammonia solution to 2 ml of hydroxylate, shaking vigorously before adding 3 ml of chloroform to separate the chloroform layer.

**Protein identification**

To 500µl of extract, 5ml of Bradford solution is incorporated, nurtured at the darkest place for 10 to 15 mins, and noted down the O.D. at 575 nm.

**Phenol identification**

Fifty milligrams of extract include five milliliters of distilled water with fewer 5% ferric chloride droplets. Dark green coloration that results from the production of phenol.

**Saponinidentification**

To the 50 mg of extract, 20 ml of distilled $H_2O$ is included and shaken rapidly for 15 mins. The creation of a 2 cm coating of foam indicates the existence of saponins.

**Alkaloids - Mayer's test**

Two drops of Mayer's solution are placed across the edges of the test tube to a few ml of plant specimen extracts; the formation of a white, milky residue confirms the existence of alkaloids.

**Saponification test**

The presence of soap or fat after boiling 1 or 2 ml of 10 N NaOH with 2 ml of extraction for 2 minutes indicates saponification.

**Gum Test**

In 2 ml of distilled water, 100 mg of plant extract is dissolved, and the mixture is then added to 2 ml of pure alcohol while being constantly stirred.

**Flavanoglycoside identification**

The creation of pink suggests the existence of flavanoglycoside. Furthermore, 50 mg of plant material is diluted in 5 ml ethanol. Fewer droplets of magnesium sulfate and concentrated HCL were also added.

**Carbohydrates identification**

In 0.5 ml of extract, Benedict reagent is introduced, and the mixture is heated for 2 minutes. Changes in hue and precipitate creation both happen. It indicates the presence of carbohydrates.

**Resins identification**

Add 3 ml of $CuSO_4$ solution to 0.5 ml of extract from plants. The green residue that forms after being shaken for around 1-2 minutes suggests the existence of resins.

**Biurette test**

To 2 ml of extract, one droplet of 2 percent $CuSO_4$ solution, and then pour 1 ml of 95 percent ethanol, followed by adding 2 to 3 NaOH pellets. The development of a pinkish color denotes the test positive.

**Protease inhibition assay**

The Oyedepo and Femurewa-modified technique for the proteinase inhibiting test is used. The reaction mixture (2 ml) comprised varied concentrations of the AI01 specimen (500, 250, 100, 50, and 10 g/ml), 0.06 mg trypsin, and 1 ml of

Tris-HCl buffer (20 mM, pH 7.4). After 5 mins of incubation at 37 °C, 1 ml of 0.8 percent casein is mixed into the reaction mixture. The mixture is incubated for a further 20 minutes. Two ccs of 70% perchloric acid are added to stop the reaction. The supernatant's absorption at 210 nm is estimated using a Tris-HCl solution as a reference following centrifuging in the cloudy suspension. The study is performed three times.

Inhibition percentage = [(absorbance of control- absorbance of the reaction mixture)/absorbance of control] X 100.

**Anti-inflammatory action**

When an outside force or substance, like a powerful acid or base, when a concentrated mineral salt, an organic solvent, or heat is employed, proteins break down their tertiary and secondary structures, a procedure referred to as denaturation. When denatured, almost all biological proteins stop working functionally. Inflammation has been linked to the denaturation of proteins, which is widely known. The potential of plant extract to suppress protein denaturation is investigated as part of the inquiry into the mode of the anti-inflammation action. It is successful in stopping heat-generated albumin denaturation. The denaturation of proteins is the primary reason for infection. Protein denaturation suppression is assessed. Acetylsalicylic acid is used as a positive control. 500 µL of 1 percent bovine serum albumin is incorporated into the LCZ of the test specimen. This combination is placed at room temperature for 10 mins, then boiled at 51°C for 20 mins. The study is performed in triplicates, and the % of inhibition for protein denaturation is determined by:

Percent Inhibition=100− ((A1-A2)/A0) *100)

A1 indicates the absorbance of the control, A2 denotes the absorbance of the test specimen, and A0 represents the absorbance of the positive control.

All experiments and studies were performed in triplicate and summed to calculate the $IC_{50}$ values, where $IC_{50}$ is characterized as the concentration required for attaining 50 percent of a maximal scavenging capability.

**Results**

The results of a qualitative study of phytochemicals for all three plant extracts LCZ results are exemplified in Table 1. A qualitative study of LCZ confirmed the existence of resins, carboxylic acid, steroids, flavonoids, carbohydrates, saponification, and gum in all three extracts. These substances were determined to be present in the extract with a very lesser amount (+). Tannin, glycoside, phenol, biuret, saponin, flavanoglycoside, and alkaloids are absent (-) in LCZ extracts. Overall, the protein component was found to be present in a moderate amount (++) in the LCZ extracts.

Table 1. A qualitative study of plant extracts.

| S.No | Name of the Specimen | Phytochemical substance | Result |
|---|---|---|---|
| 1. | LCZ | Resins | + |
| 2. | | Carboxylic acid | + |
| 3. | | Tannins | - |
| 4. | | Steroids | + |
| 5. | | Flavonoid | + |
| 6. | | Carbohydrates | + |
| 7. | | Glycosides | - |
| 8. | | Saponification | + |
| 9. | | Protein | ++ |
| 10. | | Phenol | - |
| 11. | | Biuret | - |

| | | | |
|---|---|---|---|
| 12. | | Saponin | - |
| 13. | | Gum | + |
| 14. | | Flavanoglycoside | - |
| 15. | | Alkaloids | - |

**Note:** ++: moderately present, +: Low, -: Absent.

**Protease inhibition assay**

The aqueous extracts of LCZ at various concentrations provided significant inhibition of proteinase assay. The maximum absorbance was observed in control with a value of 0.426 whereas the LCZ extracts showed a nearly similar absorbance value of 0.367 at a concentration of 10 µg/ml. Likewise, at a concentration of 50 and 100 µg/ml extracts displays the absorbance value of 0.293 and 0.287. The least absorbance value was identified as 0.261 at an LCZ concentration of 500 µg/ml. From these results, it was clear that the absorbance value is totally concentration dependent where the decreased concentration undoubtedly shows the increased absorbance value. Hence, the maximum absorbance was observed at 10 µg/ml moderately similar to that of the control group (Figure 2).

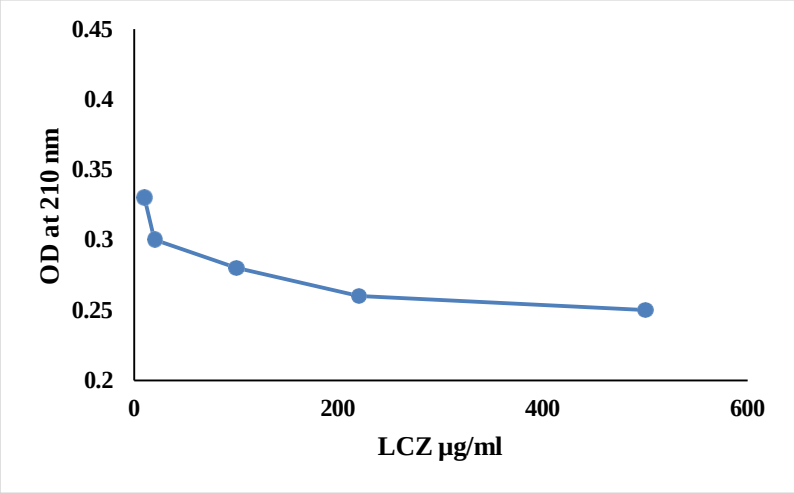


Figure 2. The absorbance value of LCZ extract at different concentrations for Protease inhibition assay

In this protease inhibitory assay, trypsin degraded casein through its proteolytic activity. Here, trypsin is employed as a serine protease. The deterioration of casein is decreased through the inhibition of trypsin by the substances that exist in the LCZ extracts. The protease inhibition activity of the LCZ extract and controlimproved linearly in a concentration-dependent pattern. For instance, the inhibition by the LCZ extract is concentration dependent with 10 µg/mL which has an inhibition of 0 %, and 500 µg/mL with the highest inhibition of 37.4864 % (Figure 3). Maximum inhibition % at 500 µg/ml concentration of LCZ extracts was found to be 37.4864 % slightly similar to that of 250 µg/ml concentration of LCZ (34.0419%).

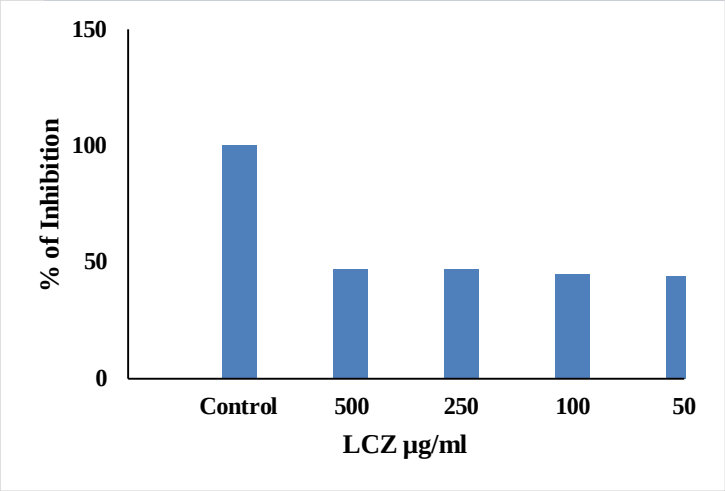


Figure 3. Percentage of inhibition for Protease inhibition assay

**Anti-inflammatory activity**

The Anti-inflammatory activity of the LCZ extract was evaluated towards denaturation of the egg albumin process. The process by which proteins become denaturized is unexpected and involves changes to hydrophobic, disulfide, and electrostatic hydrogen bonds.Protein denaturation, a process that occurs when proteins are subjected to external stressors or chemicals, is thought to be a sign of arthritic and inflammatory conditions.The presence of phytochemical-rich contents in the extract of LCZ to impede protein denaturation also confirms its anti-inflammatory activity.The maximum absorbance was observed in control with a value of 0.702 whereas the LCZ extracts showed a nearly similar absorbance value of 0.407 at a concentration of 10 µg/ml. Likewise, at a concentration of 50 and 100 µg/ml extracts displays the absorbance value of 0.219 and 0.186. The least absorbance value was identified as 0.158 at an LCZ concentration of 500 µg/ml. From these results, it was clear that the absorbance value is totally concentration dependent where the decreased concentration undoubtedly shows the increased absorbance value. Hence, the maximum absorbance was observed at 10 µg/ml with a value of 0.284 (Figure 4).

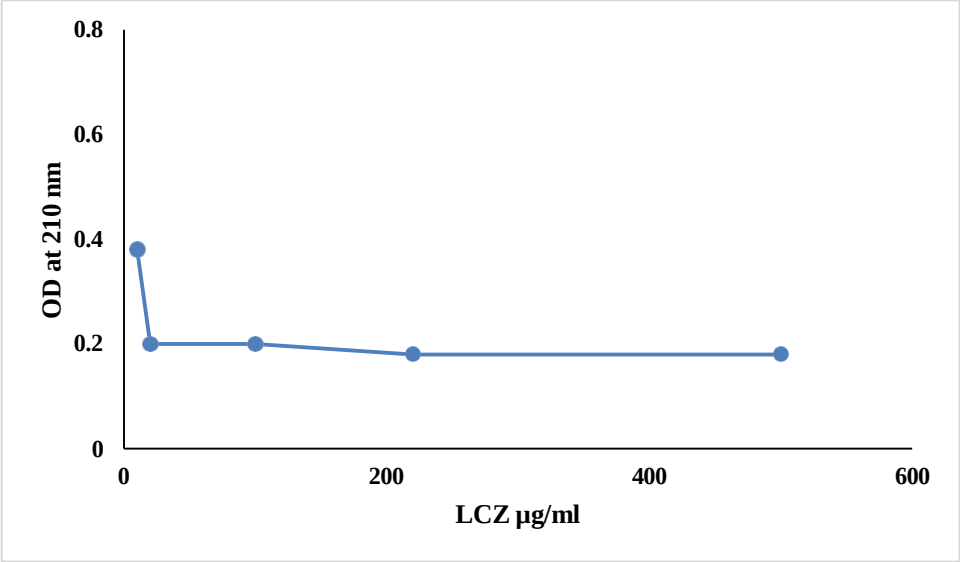


Figure 4. The absorbance value of LCZ extract at different concentrations for albumin denaturation assay.

The highest inhibition rate was observed in the LCZ extracts at the concentration of 500 µg/ml. Moreover, the inhibition

by the LCZ extract is concentration dependent with 10 µg/mL which has a lesser inhibition of 25.13 %, and 500 µg/mL with the highest inhibition of 80.91 % (Figure 5). Maximum inhibition % at 500 µg/ml concentration of LCZ extracts was found to be 80.91 % slightly similar to that of 250 µg/ml concentration of LCZ (79.03%).

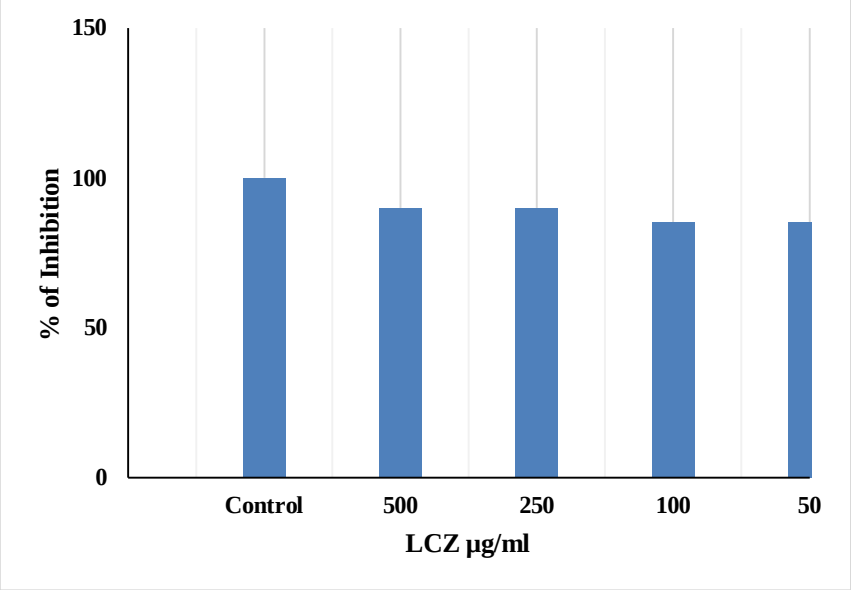


Figure 5. Percentage of inhibition for albumin denaturation assay.

**Discussion**

Medicinal plants show a significant contribution to the pharmaceutical and farming fields. World Health Organization describes herbal-based medicines as, generally happening, plant-sourced materials with next to zero modern handling, which have been employed to improve illness within conventional therapeutic practices. *Coccinia Grandis, Leucas Aspera, and Zingiber Officinale* are commonly used in Asia's Ayurvedic, Siddha, and Unani conventional medical practices. Different parts of the plant material have historically been employed in traditional medicine for diverse functions, like anti-diabetic, anti-bacterial, anti-inflammatory, anti-oxidant, anti-malarial, anti-dyslipidemic, anticancer, etc. Ginger treats a variety of illnesses by noting complications or related symptoms in many disorders.

The existence of these phytochemicals might be suggestive of various valuable health impacts, which have been mentioned in various studies. Various plant chemicals and biological compounds are derived from medicinal plants. This study focuses on finding the most potent plant extracts and exhibiting their effectiveness of extracts. In the present study, the screening for phytochemicals showed the presence of flavonoids, carbohydrates, saponification, protein, resins, carboxylic acid, gum, and Steroids. The LCZ sample is analyzed in the protease inhibition assay, and the higher (500µg/ml) concentration of the sample mean value is 37.4864 compared to the lower(10µg/ml) concentration means a value of 0. The $IC_{50}$ value in a sample was 68.56 µg/ml in the protease inhibitory assay. Again, the LCZ sample was analyzed in the anti-inflammatory action, suppression of albumin denaturation assay, and the higher (500µg) concentration of the sample mean value is 80.9151 compared to the lower (10µg) concentration mean value is 25.1392. The $IC_{50}$ value of the LCZ sample was identified as 42.66µg/ml by employing an albumin denaturation assay.

**Conclusion**

As a form of supplementary medicine, medicinal plants, and their secondary metabolites are increasingly employed to treat disease. Numerous diseases, including rheumatic and immune-mediated ailments, diabetes, cardiovascular accidents, and other conditions, are included in the broad category of pathologic states known as inflammation. We list a few plants whose anti-inflammatory properties have been proven in both clinical and experimental research. The findings backed up the plant's historic use for several painful and inflammatory illnesses. It is hypothesized that one of the constituents, or the combination of them all, is what causes the analgesic and anti-inflammatory effects because the

same plant contains biologically active elements like flavonoids, tannins, phenolic compounds, and phytosterols.The study's findings, as mentioned above, imply that plant material in all forms has strong anti-inflammatory properties. The beneficial properties of LCZ extract may be due to the existence of most of all phytochemicals. It also expresses hope for the creation of more plant-based anti-inflammatory, -fungal, -viral, -oxidant, -malarial, -dyslipidemia, -cancer, analgesic, antipyretic, -tussive, -nociceptive, hepato- and neuro-protective function medicinal products in future generations.

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**Conflict of interest statement**
"Each author declares that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangement, etc.) that might pose a conflict of interest in connection with the submitted article."

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