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## A pharmacological evaluation of ethanol extract of alpinia calcarata rhizome for anti-oxidant, anti-inflammatory and anti-asthmatic properties

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Article History	Abstract
Article History Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 12 Oct 2023	Abstract Alpinia calcarata rhizome ethanolic extract was tested for anti-asthmatic, antioxidant, and anti-inflammatory properties. Adaptogens normalize leukocytosis after milk consumption. Eosinophils are necessary for allergic illness development. The plant extract significantly reduced allergic asthma- related eosinophil cell count compared to the control group. Eosinophil count decreases cell recruitment and IL-4, IL-5, and IL-13, which affect cell count. Studies on milk-induced leukocytosis and eosinophils verified the plant extract's anti-asthmatic capabilities. In guinea pigs, goats, horses, dogs, and humans, histamine contracts trachea and bronchial muscles. Tracheas in guinea pigs test asthma drugs. The isolated guinea pig trachea contracts dose- dependently after H1 receptor stimulation. Alpinia calcarata reduced histamine-induced trachea constriction in solitary guinea pigs, proving its anti-asthmatic and H1 receptor antagonist capabilities. Hydrogen peroxide scavenging and reduction were used to test antioxidants. A hydrogen peroxide-scavenging Alpinia calcarata rhizome ethanol extract. Hydrogen peroxide scavenged less than ascorbic acid. Increasing Alpinia calcarata rhizome ethanolic extract dramatically lowered power. In vitro, ethanolic Alpinia calcarata rhizome extract stabilized rabbit red blood cell membranes and prevented protein denaturation. The ethanolic Alpinia calcarata rhizome extract was anti-asthmatic. Antioxidant and anti-inflammatory characteristics aid the plant's anti-astatic effects. Most asthma medications are steroidal. The phytochemical study identified steroids and flavonoids. Chemical moieties may make the plant anti acthmatic. The findines support the conventional and hytochemical study identified steroids and flavonoids. Chemical moieties
CC License	advise more anti-asthmatic active component study.
СС-Б I -INC-ЗА 4.0	<b>Keywords:</b> Ethanol extract, alpinia caccarata rhizome, anti-asthmatic,
	anti-oxidant, anti-inflammatory

## 1. Introduction

Long before prehistory, plants were employed as medicine. Ancient Egyptian papyrus, Chinese, and Unani scrolls mentioned plants. Herbal medicine has been used by Unani Hakims, Indian Vaids, Europeans, and Mediterraneans for about 4000 years. Traditional medicinal systems like Unani, Ayurveda, and Chinese Medicine meticulously use herbs, while indigenous cultures like Rome, Egypt, Iran, Africa, and the Americas utilised plants in healing rituals. Traditional medicinal properties of plant materials as a result of a number of factors, including rising global population, insufficient drug supply, high treatment costs, negative side effects of some synthetic treatments, and drug resistance in infectious diseases. India was rich in medicinal herbs in ancient times. The Indian Forest is the main source of medicinal and aromatic plants used to make medications and perfumes. INDIA has codified 8,000 herbal treatments in AYUSH systems. Indigenous medicine includes Ayurveda, Unani, Siddha, and Folk (tribal). India's most established and practised systems are Ayurveda and Unani Medicine [3-5].

WHO estimates that 80% of people globally use herbal medicines for primary health care. WHO estimates 21,000 plant species can be utilised medicinally. Over three-quarters of the world's population relies on plants and plant extracts for health care. More than 30 percent of plant species were used in treatment. In developed nations like the United States, botanical pharmaceuticals account for 25 percent of the market. But 80% in fast-developing countries like India and China. Thus, medicinal plants are more important to India than the rest of the globe economically. These countries supply two-thirds of modern medicine's plants, and rural health care relies on indigenous medicine [6-8]. No or few side effects make medicinal plant treatment safe. The major benefit is that these medicines match nature. The best part is that herbal remedies work for all ages and genders. Ancient scholars only believed plants could treat certain health issues and diseases. They studied and tested to determine the usefulness of medicinal herbs. This method typically results in safe and effective drugs. Herbal medicine is becoming more popular because of this. These medicinal herbs offer sensible treatment for many interior disorders that are difficult to treat [9, 10].

Aloe, Tulsi, Neem, Turmeric, and Ginger treat common diseases. Many states consider them home treatments. Many people use Basil (Tulsi) to make medicines, black tea, pooja, and other daily activities. Herbs are used as symbols of luck in many countries to commemorate their kings. Many buyers grew tulsi and other medicinal plants after reading about their health benefits. Medicinal plants are rich in elements for pharmacopoeial, non-pharmacopoeial, and synthetic medication production. Also, these plants are crucial to the development of human cultures worldwide. Some plants are nutritious and suggested for their medicinal properties. These plants include ginger, green tea, walnuts, aloe, pepper, and turmeric. Some plants and their derivatives provide active components for aspirin, toothpaste, etc [11-13].

Besides medicine, herbs are utilised in natural dye, pest control, cuisine, perfume, tea, and more. Many countries utilise medical plants and herbs to keep ants, flies, mice, and other pests away from homes and offices. Pharmaceutical manufacture now relies on medicinal botanicals. Effective remedies for conditions like diarrhoea, constipation, high blood pressure, low sperm count, dysentery, weak penile erection, piles, coated tongue, menstrual disorders, bronchial asthma, leucorrhoea, and fevers can be found in the recipes provided by traditional medicine practitioners [14-16]. There has been a lot of interest in herbal medicine during the past two decades, but not a lot of studies into it. The World Health Organisation published three volumes of monographs on various medicinal plants in 1999.

## 2. Materials And Methods

#### **Selection of Plant**

Alpinia calcarata was chosen for this investigation due to its traditional applications. Rhizomes were used.

#### Selection of Animals

Work was done by Swiss albino mice weighing 25-40 gm and by Guinea pigs weighing 400-600 gm. Animals were given free access to commercial feed and water. Animals were housed in standard

cages with a 12:12 light/dark cycle and temperatures between 25 and 20 degrees Celsius. The experiments were conducted in accordance with the standards established by the CPCSEA in New Delhi, India.

#### Plant collection and identification

The dried Alpinia calcarata rhizomes were obtained. A motorised grinder ground the rhizomes into coarse powder after cleaning and shade drying.

#### **Extraction of plant material**

The soxhlet extractor extracted powdered rhizomes with ethanol. The extractor with ethanol dissolves the powdered medication. Evaporation condensed the extract, which was refrigerated for use.

#### **Acute Toxicity Studies**

Alpinia calcarata acute toxicity was assessed using OECD standards 423. A specially developed mice oral tube was used to gauge the chemical in one dose. Fasted animals were given food but not water overnight before treatment. After fasting, the animals were weighed and given 5, 50, 300, and 2000 mg/kg of the test drug orally. Animals are watched for toxic symptoms such increased motor activity, salivation, violent convulsions, coma, and death over the first three hours. For 24 hours, observe how an injection affects animal behaviours. 14 days of observation are required for treated animals. The 1/10th or 1/20th dose was tested to see if the extract kills at the greatest dose.

#### **Evaluation of the Anti-Asthmatic Activity**

#### In vivo anti-asthmatic activity

In guinea pigs, histamine aerosol caused bronchoconstriction: 0.2% weight/volume of histamine was dissolved in distilled water. Histamine aerosol was inhaled into guinea pigs using an aerosol chamber nebulizer, which resulted in bronchial asthma. Each animal's histamine-induced preconvulsive dyspnoea period was recorded. In the histamine chamber, 0.2% histamine aerosol was administered to each animal. Aerosol exposure to dyspnea and convulsion were used to determine the preconvulsion time (PCT). The animals were taken out of the chamber and placed outside to recover when preconvulsion dyspnoea (PCD) was noticed. The time of the initial preconvulsive dyspnea was noted. Two days were given to guinea pigs to recover from dyspnoea. The animals were then divided into four groups of 4-5. Control animals in group 1 got carboxy methyl cellulose. Group 2 and 3 received 100 and 200 mg/kg of the plant extract orally via intubation, whereas group 4 received chlorpheniramine maleate intraperitoneally. To determine pre-convulsive time, all animals were subjected to histamine maleate in the chamber for one, four, and 24 hours after receiving the medicines.

Leukocytosis and eosinophilia were caused by milk: Each of the four groups had six mice. Blood was taken from the area behind the eye. As a control, group 1 got a solution of carboxy ethylcellulose. Groups 2 and 3 got 100–200 mg/kg of plant extract, and group 4 got 50 mg/kg of i.p. dexamethasone. All of the groups gave s.c. 4 ml/kg of hot and cold milk 30 minutes after the treatments. Before the test chemical injection and 24 hours after the milk injection, leukocyte and eosinophil numbers were taken from each group. Difference between the total number of leukocytes and eosinophils before and after 24 hours of medicine.

#### Ex vivo anti-asthmatic activity

Isolated preparation of the guinea pig trachea: To get tracheal tissue from Guinea pigs, their necks were broken and their carotid arteries were bled. The trachea was cut open, put in a Krebs solution dish, and cut in the middle of each piece of cartilage. It had a hole in it and was kept at  $37 + 0.5^{\circ}$ C. The delayed trachea was given at least 40 minutes to settle down. At balance, 15 minutes of Krebs solution were added to the bath. A dose response graph was made for 10 g/ml of histamine in Krebs solution and 1 mg/ml of plant extract in Krebs solution. A histamine dosage response curve was made by plotting the percentage of highest contractile response on the ordinate and the histamine concentration on the abscissa. This was done with and without plant extract.

### In Vitro Evaluation Of Antioxidant Activity Radical (Hydrogen peroxide) scavenging assay

A 20 Mm hydrogen peroxide solution was produced using standard phosphate buffer (pH 7.4). In order to remove the samples (25, 50, 100, 200, and 400 g/ml) from the distilled water, hydrogen peroxide solution (0.6 ml) was added. After 10 minutes, phosphate buffer without hydrogen peroxide was used to test hydrogen peroxide absorbance at 230 nm. Ascorbic acid used as a reference standard.

#### In Vitro Evakuation Of Anti-Inflammatory Activity Protein denaturation assay

Glacial acetic acid was used to bring the pH of a solution containing 0.2% of bovine serum albumin (BSA) to 6.8. Alcohol was used as a solvent to create test drugs with various concentrations (25, 50, 100, 200, and 400 g/ml). Using a micropipette, 50 l of each test medication were transferred to test tubes. The test tubes received 5 ml of BSA solution at 0.2% w/v. The control consists of 5 ml of alcohol and 0.2% (w/v) BSA solution. After being heated for five minutes at 720 C, the test tubes were chilled for ten minutes. Using a UV-visible spectrophotometer set at 660 nm, the absorbance of these solutions was calculated. For the purpose of determining absorbance, diclofenac sodium was employed as the reference substance. The following formula was used to determine the percentage of protein denaturation inhibition.

#### **Statistical Analysis**

The statistical analysis was done using one-way analysis of variance (ANOVA) and Dunnett's multiple comparison test. The results are shown as MeanS.E.M. with a n=6.

#### Anti Asthmatic Activity Evaluation

#### In vivo evaluation of anti asthmatic activity

#### In guinea pigs, histamine aerosol caused bronchoconstriction

In the current investigation, guinea pigs with histamine-induced bronchoconstriction were used to test the anti-asthmatic effect of an ethanolic extract of Alpinia calcarata rhizomes. When exposed to histamine at a dose of 200 mg/kg over the course of four hours, the plant's ethanolic extract significantly prolonged the latent period of convulsion compared to the usual medication. The latent period of convulsion was used to calculate the protection percentage. The plant's ethanolic extract was calculated to provide 60.79% of maximal protection at a dose of 200 mg/kg. Chlorpheniramine maleate, the usual medication, demonstrated notable% protection at times 1 hour and 4 hours. The plant extract at a concentration of 100 mg/kg demonstrated 43.2% protection at time 1 hour, 40.2% protection at time 24 hours, and 57.2% protection at time 4 hours. The control, carboxymethyl cellulose, produced protection levels of 10.9% after an hour, 12.3% after four hours, and 11.4% after twenty-four hours. The results of the plant extract at 200 mg/kg were 48% at time 1, 60.79% at time 4, 44.3% at time 4, and 44.3% at time 24 hours. Chlorpheniramine maleate, a common medication, offers 69.76% protection after one hour, 78.3% after four hours, and 50.1% after twenty-four hours. Table 1 and Figure 1 illustrate the outcomes of histamine aerosol-induced bronchoconstriction in guinea pigs.

Crown -	Crown		Latent period	
Group	Before 1 hr	1 hour	4 hour	24 hour
Control Group	$15.9 \pm 2.32$	19.04±0.12	19.44±0.190	17.95±0.15
Ethanolic extract of <i>Alpinia</i> calcarata (100 mg/kg)	15.99±1.54	30.11±0.32	38.99±0.05*	27.12±0.25
Ethanolic extract of <i>Alpinia</i> calcarata (200 mg/kg)	16.10±0.99	31.2±2.99	41.11±1.04*	29.5±.35
Standard Group (CPM) (1 mg/kg)	19.14±0.94	$59.90 \pm 0.02^*$	69.20±1.01**	37.10±0.70

**Table 1:** In guinea pigs, histamine aerosol caused bronchoconstriction

Values are Mean S.E.M., with n = 6 for each group, and are significant (P 0.05\*, P 0.01\*\*) when compared to the control. One-way analysis of variance (ANOVA) and Dunnett's multiple comparison test were used for the statistical study.



Figure 1: Ethanolic extract of Alpinia calcarata rhizomes on histamine-induced airway constriction in guinea pigs

**Table 2:** Rhizomes of Alpinia calcarata prevent histamine-induced bronchoconstriction in guinea pigs by %

Crown	% Protection		
Group	1 hour	4 hour	24 hour
Control group	11.0	11.30	12.40
Ethanolic extract of Alpinia calcarata (100 mg/kg)	42.90	58.30	39.20
Ethanolic extract of Alpinia calcarata (200 mg/kg)	48.21	51.80	41.30
Standard Group (CPM)	70.11	77.30	51.10

Mean Standard Error of Mean (SEM), n=6 per group, P 0.05 \*, P 0.01 \*\* (significant) vs. control. The statistical analysis used one-way ANOVA and Dunnett's multiple comparison test.





#### Milk induced eosinophilia

Differences in eosinophilic count before and after treatment were studied. The control group had the highest eosinophilic count increase ( $120\pm1.45$ ). The ethanolic Alpinia calcarata extract (200 mg/kg) significantly reduced eosinophilic count ( $54 \pm 1.44$ ). The usual medicine significantly reduced eosinophilic count by  $39 \pm 1.14\%$ . The 100 mg/kg plant ethanolic extract proved inactive. The results were in table 4 and figure 3.

Groups	Difference in no of leukocytes before and after treatment(Cu.mm)	
Control Group	4101±90	
Ethanolic extract of Alpinia calcarata (100 mg/kg)	$2620\pm8^*$	
Ethanolic extract of Alpinia calcarata (200 mg/kg)	$1270 \pm 12^{**}$	
Standard Group Dexamethasone (50 mg/kg)	$610{\pm}10^{**}$	

Table 4: Ethanolic Alpinia calcarata rhizome extract on milk-induced eosinophilia
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Mean Standard Error of Mean (SEM), n=6 per group, P 0.05 \*, P 0.01 \*\* (significant) vs. control. The statistical analysis used one-way ANOVA and Dunnett's multiple comparison test.



Figure 2: Rhizomes of Alpinia calcarata affect milk-induced leukocytosis

#### Milk induced eosinophilia

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Table 4: Ethanolic Alpinia calcarata rhizome extract on milk-induced eosinophil
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Groups	Difference in no of eosinophilic count before and after treatment(Cu.mm)
Control (Carboxy methyl cellulose)	120±1.45
Alpinia calcarata ethanolic extract (100 mg/kg)	$83{\pm}1.2^{*}$
Alpinia calcarata ethanolic extract (200 mg/kg)	$54{\pm}1.44^{**}$
Standard (Dexamethasone (50 mg/kg))	39±1.14**

Mean $\pm$  SEM, n=6 per group, P< 0.05 \*, P< 0.01 \*\* (significant) vs. control. Dunnett's multiple comparison test and one-way ANOVA were used for statistical analysis.





#### *Ex vivo* anti-asthmatic study

#### Isolated guinea pig tracheal preparation

Histamine (10 g/ml) caused a dose-dependent narrowing of the guinea pig tracheal preparation. The rhizome ethanolic extract of Alpinia calcarata significantly decreased histamine's contractile activity when used as a pretreatment. Plant extract concentration response curve in guinea pig tracheal preparation pre- and post-administration. Table 5 summarizes the findings, and Figure 4 displays the impact of Alpinia calcarata rhizomes on histamine-induced tracheal contraction in solitary guinea pigs.

**Table 5:** Ethanolic Alpinia calcarata rhizome extract on histamine-induced tracheal constriction in isolated guinea pigs

Dose of histamine (10µg/ml) in ml	Control (Histamine 10 µg/ml) % maximum contraction	Test Histamine(10µg/ml) + EEAC (1mg/ml) % maximum contraction
0.10	$39.46 \pm 1.60$	$31.90 \pm 1.32^{**}$
0.20	$54.50 \pm 4.26$	$47.20 \pm 2.89^{**}$
0.40	$62.50\pm3.90$	$54.60 \pm 3.29^{**}$
0.80	$74.08 \pm 2.34$	$66.0 \pm 1.78^{**}$
1.60	85.00 ± 2.41	$70.20 \pm 1.10^{**}$
3.2	$101 \pm 1.070$	$77.90 \pm 2.30^{*}$

Mean $\pm$  S.E.M. values, n=6 per group, P< 0.05 \*, P< 0.01 \*\* (significant) vs. control. Dunnett's multiple comparison test and one-way ANOVA were used for statistical analysis.



Figure 4: Ethanolic Alpinia calcarata rhizome extract on histamine-induced tracheal constriction in isolated guinea pigs

### In Vitro Anti-Inflammatory Activity

#### **Protein denaturation**

Protein denaturation was used to examine whether or not a plant extract has anti-inflammatory effects. Protein denaturation caused by heat was halted. Diclofenac sodium, a common anti-inflammatory drug, blocks inflammation to a great extent. At 200 and 400 g/ml, the ethanolic extract of Alpinia calcarata rhizome shows significant inhibitory action.

Concentration (µg/ml)	Absorbance [A]	% inhibition
25	$1.30\pm0.06$	14.1
50	$0.58 \pm 0.04$	61.60
100	$0.39 \pm 0.01$	75.00
200	$0.19 \pm 0.01$	87.40
400	$0.18 \pm 0.002$	89.00
Diclofenac sodium (100µg/ml)	0.17±0.01	89.00

Table 6: Protein denaturation with Alpinia calcarata ethanolic extract

Mean $\pm$ S.E.M. values (n=6) in each group, P< 0.05 \*, P< 0.01 \*\* (significant) compared to control. One-way ANOVA and Dunnett's multiple comparison test were used for statistical analysis.



Figure 5: Effect of Alpinia calcarata ethanolic extract on protein denaturation

Alpinia calcarata rhizome ethanolic extract phytochemical composition was examined. Rhizome extract contained phenol, tannins, cardiac glycosides, flavonoids, proteins, and carbohydrates. Plant chemical components synthesize complex compounds and evaluate biological activity. Plants contain bioactive phytochemicals. Main and secondary metabolites. Secondary metabolites include alkaloids, terpenoids, and phenolic chemicals; main metabolites are carbohydrates, amino acids, proteins, and chlorophyll. Plant secondary metabolites have medical benefits. Thus, finding new bioactive chemicals requires extensive plant species screening [17, 18]. Phytochemicals like flavonoids are antioxidant secondary metabolites. Their biological mechanisms include heart protection, cell growth, and anti-aging. Tannins damage plant viruses, bacteria, and fungi. Research reduces their risk of coronary heart disease. The study validated the plant's bronchodialotor characteristics, aiding asthma treatment [19, 20].

An ethanolic extract of Alpinia calcarata rhizomes (100, 200 mg/kg) was investigated for asthma treatment in mice with milk-induced leukocytosis and eosinophilia. Many mediators induce asthma. After 24 hours, milk administration boosted leukocytes and eosinophils. Leukocytes perpetuate asthmatic inflammation with cytokines, histamine, and major basic protein. Leukocyte infiltration enhances inflammation, oxidative stress, and asthma by producing reactive oxygen species [21]. Leukocyte counts were considerably lower in mice treated with plant ethanolic extract at 100 and 200 mg/kg than the control group. Ethanolic Alpinia calcarata rhizome extract decreases milk-induced leukocyte count via normalizing oxidative stress. An abnormal increase in peripheral eosinophil to more than 4% of total leukocytes is called "eosinophil" Eosinophilic counts, mucus hypersecretion, and airway hyperreactivity are higher in asthmatics. Airway eosinophils affect goblet cell mucus production. Our investigation found that 100 and 200 mg/kg Alpinia calcarata rhizome ethanolic extract inhibited milk-induced eosinophils. By decreasing eosinophils and leukocytes and restoring oxidative stress, Alpinia calcarata rhizome ethanolic extract reduces asthma type I hypersensitivity. Thus, Alpinia calcarata rhizomes' ethanolic extract relieves asthma [22, 23].

An ethanolic Alpinia calcarata rhizome extract reduces histamine-induced tissue contraction. Histamine (10 g/ml) dosages created a concentration response curve. The study found that Alpinia calcarata rhizome ethanolic extract at 0.8 mg/ml significantly reduces contracture. The ethanolic Alpinia calcarata rhizome extract dose-dependently scavenged hydrogen peroxide. H2O2, a physiologically significant non-radical oxidant, is produced through tissue oxidation. The weak oxidant hydrogen peroxide can directly inactivate certain enzymes by oxidizing crucial thiol (-SH) groups. Hydrogen peroxide is not reactive, but it can start a fenton reaction in cells that produces a

hydroxyl radical (OH) that peroxidizes lipids. Thus, cellular or dietary antioxidant defense requires H2O2 elimination. Hydrogen peroxide oxidizes cell membrane components. Extracts quench OH- to avoid lipid peroxidation and slow chain reaction by scavenging active oxygen species. The Alpinia calcarata rhizome ethanolic extract had hydrogen peroxide activity compared to 100 g/ml ascorbic acid [24, 25].

Protein denaturation is linked to inflammation in numerous studies. Autoantigens may result from tissue protein denaturation. Anti-inflammatory drugs that prevent protein denaturation may be helpful. Denaturation likely alters electrostatic hydrogen, hydrophobic, and disulfide bonds. Some non-steroidal anti-inflammatory medicines stabilize heat-treated protein at physiological pH [26, 27]. Concentration-dependent protein denaturation was inhibited by Alpinia calcarata rhizome ethanolic extract. The rhizome plant extract showed substantial in vitro anti-inflammatory properties. A plant's ethanolic extract stabilizes membranes by suppressing hypotonicity, boosting anti-inflammatory effects. Cell vitality depends on RBC membrane integrity. Hypotonic medium causes hemolysis, membrane lysis, and hemoglobin oxidation. Early inflammation is prevented by membrane-stabilizing drugs. Zingiberaceae perennial Alpinia calcarata is rhizomatous. Traditional therapies included asthma, bronchitis, rheumatoid arthritis, stomachic ailment, diabetes, and heart disease [28, 29].

Alpinia calcarata rhizome ethanolic extract is phytochemically and pharmacologically studied. Ethanol-soxhlet-extracted powdered rhizomes. In early phytochemical screening, carbohydrates, cardiac glycoside, protein, alkaloids, steroids, flavonoids, tannins, and phenols were found. Ingredients may reflect plant biology. The 2000 mg/kg ethanolic Alpinia calcarata rhizome extract was not harmful. The most clinically and economically burdensome asthma is allergic and inflammatory. Airway disease asthma. Smooth muscle spasm and histamine production cause bronchial asthma blowout constriction. A hydrogen peroxide-scavenging Alpinia calcarata rhizome ethanol extract. Hydrogen peroxide scavenged less than ascorbic acid. Increasing Alpinia calcarata rhizome extract stabilized rabbit red blood cell membranes and prevented protein denaturation [28-30].

#### 4. Conclusion

The study found anti-asthmatic activity in Alpinia calcarata rhizome ethanolic extract. The plant's antioxidant and anti-inflammatory properties support its anti-asthmatic properties. Most asthma medications are steroidal. Phytochemical study revealed flavonoids and steroids. The plant may have anti-asthmatic properties due to these chemical moieties. The findings support the conventional and calls for more research to extract and characterise anti-asthmatic active components. The progressive increase in the morbidity and prevalence of CKD, according to epidemiological data, highlights the importance of recognizing and understanding the specific signs and symptoms of this systemic pathology. Dental professionals must be prepared to face these challenges and appropriately and responsibly address the nursing procedures necessary in the care of these patients. Efficient and well-informed management is key to providing optimal care to those suffering from this complex kidney disease.

#### Funding

None

#### **Conflict of Interest** None

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