

# Antioxidant And Anti Inflammatory Activity Of Kabasura Kudineer, A Siddha Poly Herbal Extract

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## Abstract

### Introduction

Siddha system is one among the most common medical systems in India out of which kudineer or Kasayam is the most general form used commonly called decoction. Use of herbal medicine has increased greatly as they were easily available and less toxic and showed various properties like antioxidant, anti inflammatory etc. Kabasura Kudineer Chooranam is a traditional formulation used by Siddha practitioners for effectively managing common respiratory ailments such as the flu and cold.

### Aim

To evaluate antioxidant and anti inflammatory activity of the kabasura kudineer extract

### Materials and Methods

Kabasura kudineer was purchased from the local market and extract was prepared. Protease inhibition assay with aspirin as standard was done to evaluate anti inflammatory activity. Further it was also subjected to antioxidant activity - DPPH assay and the results obtained were statistically analysed by ONE WAY ANNOVA. The Duncan multiple range test was used to analyse the statistical difference between the groups.

### **Results and Discussion**

The results showed that as the concentration increases in a dose dependent manner the antioxidant and anti-inflammatory activity of the plant extract also increases. But since the control used has certain side effects, this can be considered a non-toxic natural alternative.

### **Conclusion**

Hence, it can be concluded that the kabasura kudineer extract has promising antioxidant and anti-inflammatory activities. Recent studies have found that it can be used against COVID-19 and SARS-CoV-2. So, more clinical trials and in vivo studies should be done for standard drug development.

**Key Words:** Novel method, Kabasura kudineer, COVID-19, antioxidant, Innovative technique

### **Introduction**

Siddha system is considered as one of the most common medical systems of India, out of which Kudineer or kashayam is considered to be the most general form to be used(1). The kudineer is prepared by boiling the drug with a specific volume of water required till it is reduced to one-fourth of the volume and then filtered. The general dosage of this kudineer that can be recommended for infants is 2.5ml, 20 ml for 3 to 5 years of age, 30ml for 5 to 12 years of age and 60-100 ml for above 12 years of age(2). The natural herbal medicines are recently used more often to treat various health problems, new-line diseases that affect the people and as a nutraceutical as it avoids various side and adverse effects and was further found to be easily available, cost-effective and less toxic(3). According to the World Health Organisation, almost 80% of the world population depend mainly on these traditional herbal medicines obtained from plants for their health care and to treat human diseases(4).

The kabasura kudineer is well known next to the nilavembu kudineer. It was primarily introduced for the prevention and management of swine flu and other respiratory diseases that are caused by the virus. It has almost 32 major phytochemical constituents thus has properties like antibacterial, antipyretic, antifungal, anti-diabetic, antiviral and various other properties(5). It is also considered to be effective against the present ongoing pandemic coronavirus and SARS-CoV-2 disease as it was proved that the phytoconstituents were free from carcinogenic and tumor properties and hence it can fight against the viruses and thereby boost the immune system of the body(6).

Antioxidants are considered to decrease the oxidative stress damage that is caused by the free radicals present in the human body. Vitamin E, lycopene etc are considered as natural antioxidants that are obtained from plants(6). Antioxidant activity was evaluated using the DPPH radical scavenging assay in the presence of these antioxidants(7). Oxidative stress is produced in the body due to the environmental and chemical factors produced in the body during the metabolic processes as it generates reactive free radicals that damage the four basic molecules such as proteins, carbohydrates, lipids and nucleic acids(8). In recent days, the need for natural antioxidants seems to be increasing when compared to synthetic antioxidants like Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT), Tertiary Butyl Hydroquinone (TBHQ) as it helps in preventing the lifestyle diseases like diabetes, cancer and other heart diseases etc(9).

Pain and fever are considered the most common symptoms that are produced by inflammation. It is prevented by using NSAIDs that prevent by modifying the inflammatory response. It does not remove the underlying cause of the disease. Though there are drugs like aspirin, indomethacin, phenylbutazone, etc. that act as effective NSAIDs they have their own limitations. These NSAIDs have both advantages and disadvantages (10) and cause various side effects like dyspepsia, gastric bleeding, ulceration etc. Hence, newer and safer drugs that are obtained from plants need to be developed to prevent these adverse side effects. They act by inhibiting the COX pathway that causes the inhibition of prostaglandin synthesis which is responsible for maintaining the gastric mucosal integrity (11). Our team has extensive knowledge and research experience that has translated into high quality publications (12), (13), (14), (15), (16), (17), (18), (19), (20), (21), (22), (23), (24), (25), (26), (27), (28), (29), (30), (31)

Hence, the present study aims in evaluating the antioxidant and anti-inflammatory properties of the Kabasura kudineer herbal extract

## **Materials and Methods**

### **Anti-inflammatory Activity: Protease inhibition assay**

Inhibition of trypsin was evaluated by the method of Oyedepo and Femure was (1965) and Sakat et al. (2010). 100  $\mu$ L of bovine serum albumin was added to 100  $\mu$ L of plant extracts (0.1 to 0.5 mg/ml) with an increase in concentrations (100-500  $\mu$ g/ml). This was incubated at room temperature for 5 minutes. Reaction was inhibited by the addition of 250  $\mu$ L of trypsin followed by centrifugation. The supernatant was collected, and absorbance was observed at 210 nm. Acetyl salicylic acid was used as a positive control. The experiment was carried out in triplicates and percent inhibition of protease inhibition was calculated. In this study, Aspirin was used as a standard anti-inflammatory drug.



**Fig 1:**The figure shows a digital calorimeter to weigh the plant extract

**Calculation:**

$$\% \text{ Inhibition} = 100 - ((A1 - A2) / A0) * 100$$

**In vitro antioxidant activity by potential of Kabasura Kudineer**

DPPH radical assay The DPPH free radical scavenging assay was performed by LiyanaPathirana and Shahidi method [Kikuzaki and Nakatan, 1993]. 200  $\mu$ L of 0.1 mM DPPH prepared in methanol was added to 100  $\mu$ L of the plant extract with an increase in concentration (100-500 $\mu$ g/ml). The resulting mixture was incubated at room temperature in the dark for 15 minutes. Absorbance was observed at 517 nm. BHT was taken as a positive control. The experiment was carried out in triplicates and percentage inhibition of the DPPH radical scavenging activity was calculated.



**Fig 2:** The figure shows digital photo colorimeter to measure the absorbance of extract at 517 nm of wavelength

### Calculation

$$\% \text{ Inhibition} = ((A_0 - A_1) / A_0) * 100$$

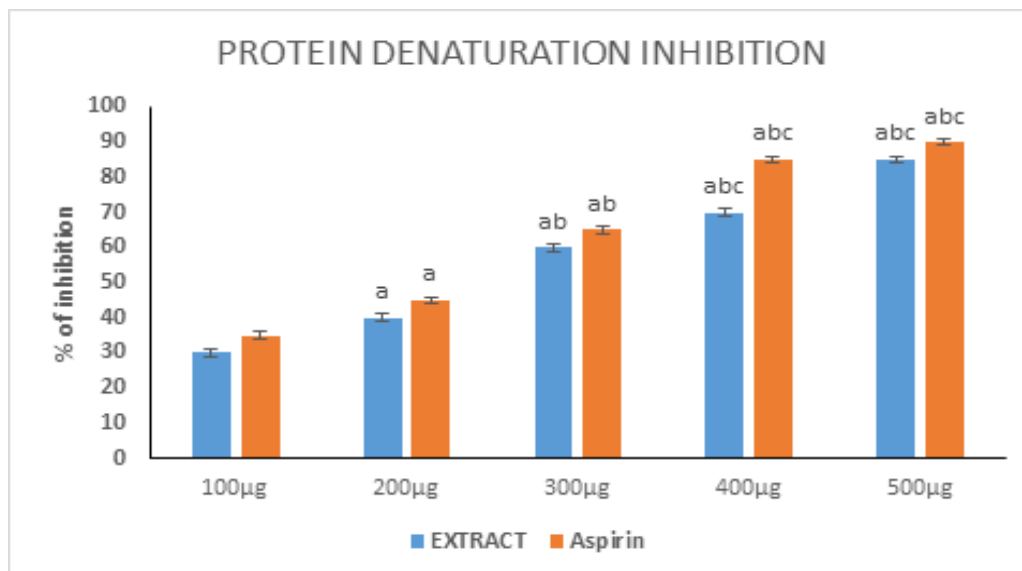
Where A0 is the absorbance of the control and A1 is the absorbance of the sample.

### Statistical analysis

The data were analysed statistically using one way analysis of variance (ONE-WAY ANOVA). Duncan Multiple range test was used to analyze the statistical significance between groups. The levels of significance were considered at the levels of  $p < 0.05$ .

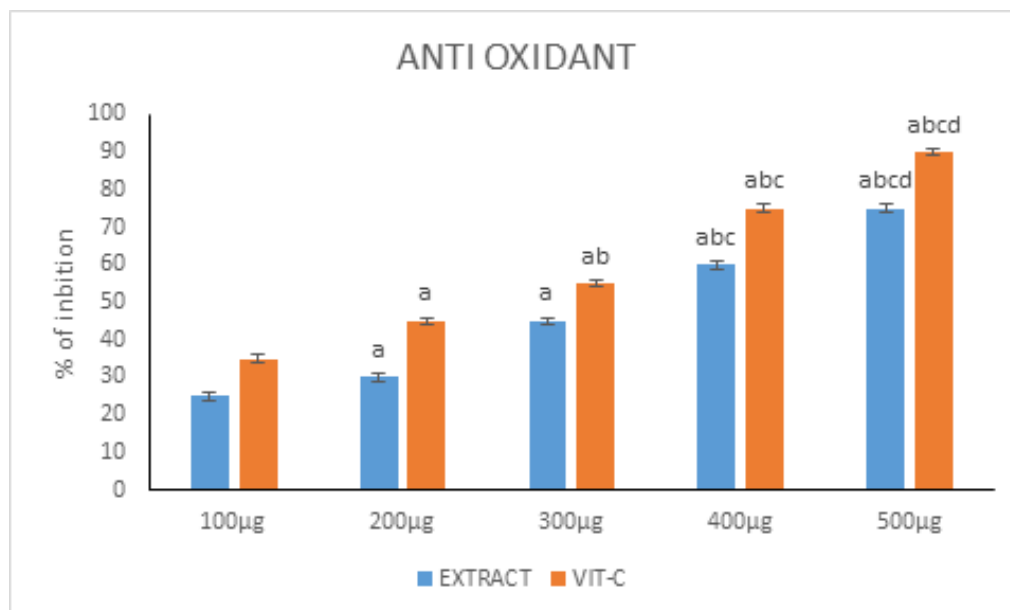
### Results and Discussion

**Anti inflammatory of kabasura kudineer extract :**



**Graph 1:** Anti inflammatory activity of kabasura kudineer extract. Each bar represents the mean  $\pm$  SD of 6 observations. Significance at the levels of  $p < 0.05$ . a-compared with 100  $\mu$ g; b-compared with 200  $\mu$ g; c-compared with 300  $\mu$ g.

**Antioxidant Activity of kabasura kudineer extract :**



**Figure 2:** Antioxidant activity of kabasura kudineer extract. Each bar represents mean  $\pm$  SD of 6 observations. Significance at the levels of  $p < 0.05$ . a-compared with 100  $\mu$ g; b-compared with 200  $\mu$ g; c-compared with 300  $\mu$ g. d-compared with 400  $\mu$ g.

The graph showed that as the concentration increased in a dose dependent manner both the anti-inflammatory and antioxidant activity of the Kabasura kudineer extract also increase but when compared to the control, the increase was found to be significantly lesser. But since the extract shows a negative correlation and the control used has certain side effects, it can be considered as a non-toxic alternative to treat human diseases.

The presence of various phytochemical constituents like tannins, phenols, flavonoids etc are found to be responsible for various properties like antioxidant, antibacterial, anti-inflammatory, antipyretic and other immunomodulatory and hepatoprotective properties(32). Flavonoids are an important group present among the polyphenols that act as the mediators of inflammation by inhibiting the prostaglandin synthesis(33). It is considered as an universal antioxidant and also called as vitamin P as it decreases the capillary permeability and fragility and considered to be non-toxic.(34). The purple colour of the DPPH was reduced to yellow. This property of antioxidants occurs due to the presence of the ability to donate hydrogen.

The Siddha system of medicine is mostly practised in South India. Its preparation can be classified either as external or internal and the kabasura kudineer choornam can be classified as an internal medicine form. Standardising the herbal formulations is very essential for assessing the quality of the drugs. The kudineer choornam contains coarse powders of drugs and used as prophylactic drug during the viral epidemics(35). The kabasura kudineer chooranam contain 15 ingredients that is used in the management of cold, cough, breathing difficulties etc. A previous study has found that the extract binds to the SARS-CoV-2 spike protein by *in silico* studies(36). The presence of the secondary metabolites are responsible for these pharmacologic activity of the polyherbal formulations. The hydrosoluble tannins is said to have astringent properties that helps in hastening the wound healing process and treating the inflamed mucous membrane(37). The saponins present in the plant can also be used as immune adjuvants in the new generation vaccine as they enhance the immune response to the antigens(38).

### **Conclusion**

Hence, it can be concluded that the kabasura kudineer extract has promising antioxidant and anti-inflammatory activities. Recent studies have found that it can be used against COVID - 19 and SARS COV - 2. So, more clinical trials and *in vivo* studies should be done for standard drug development

### **AUTHORS CONTRIBUTIONS**

Shruthi Devi R : Literature search, data collection, analysis, manuscript drafting.

Dr.Selvaraj: Aided in conception of the topic, has participated in the study design, statistical analysis and has supervised in preparation and final corrections of the manuscript.

Dr.Vishnupriya: Data verification, manuscript drafting, preparation of manuscript.

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#### **CONFLICT OF INTEREST**

The author declares that there was no conflict of interest in the present study.

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