

# Anti Inflammatory Activity of Zingiber Officinale and Panax Ginseng Individual Combined Effect on Carrageenan Induced Model in Rats

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**Abstract-** *Zingiber officinale* and *Panax ginseng*, as well-known traditional Chinese medicines, have been used together to clinically treat ulcerative colitis with synergistic effects for thousands of years. However, their compatibility mechanism remains unclear. In this study, the shift of gut microbiome and fecal metabolic profiles were monitored by 16S rRNA sequencing technology and ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry analysis, respectively, which aimed to reveal the synergistic mechanism of *Zingiber officinale* and *Panax ginseng* on the amelioration of ulcerative colitis. The results showed that the relative abundance of beneficial bacteria (such as *Muribaculaceae\_norank*, *Lachnospiraceae NK4A136* group and *Akkermansia*) was significantly increased and the abundance of pathogenic bacteria (such as *Bacteroides*, *Parabacteroides* and *Desulfovibrio*) was markedly decreased after the intervention of *Zingiber officinale*-*Panax ginseng* herb pair. And a total of 16 differential metabolites related to ulcerative colitis were identified by the metabolomics analysis, which were majorly associated with the metabolic pathways, including arachidonic acid metabolism, tryptophan metabolism, and steroid biosynthesis. Based on these findings, it was suggested that the regulation of the gut microbiota-metabolite axis might be a potential target for the synergistic mechanism of *Zingiber officinale*-*Panax ginseng* herb pair in the treatment of ulcerative colitis. Furthermore, the integrated analysis of microbiome and metabolomics used in this study could also serve as a useful template for exploring the mechanism of other drugs.

**Keywords-** *Zingiber officinale* and *Panax ginseng*, Anti Inflammatory, Carrageenan

## INTRODUCTION-

Inflammation is a defensive response which causes the different physiological adaptations which limits the tissue damage and removes pathogenic insult & Pain is an expected result of many diseases, medical care, surgical interventions and trauma. Pain is a complex experience which includes affective, cognitive and behavioral features, all of which are the result of mental process, as such; it represents psychological conditions. The phenomenon of pain, therefore, involves pathophysiological and psychological components that are frequently difficult to interpret. Suffering is a term frequently used in conjunction with pain, implying the conscious endurance of pain or distress and referring to a wide range of intense and unpleasant subjective states that may be of physical or psychological origin. The most comprehensive and exhaustive definition of pain is provided by International Association for the study of Pain, namely “an unpleasant sensation and an emotional experience associated with a real or potential damage to tissue, or equivalent of such damage. The objective of inflammation is to destroy and eliminate the damaging agent. However, if doesn't occur or is protracted process then inflammation will isolate and contain the injury. In each aspect, the objective is to allow the repair and healing of injured tissue with

the minimum damage of host's physiology. Inflammation occurs due to the stress responses and is an integral part of it.

### 1.Type of inflammation

1. Acute Inflammation
2. Chronic Inflammation

#### 1. Acute inflammation:

Acute inflammation is of short duration and its duration is from minutes to few days. The main characteristics are:

- Exudation of fluids
- Plasma protein (edema)
- Emigration of leukocytes specially neutrophils

#### 2. Chronic inflammation

Chronic inflammation is having longer duration than acute inflammation. It is associated histologically with the presence of lymphocytes, macrophages, proliferation of blood vessels, fibrosis, and tissue necrosis. It is the processes of active inflammation and tissue destruction occurs. It is followed by acute inflammation and it starts from the low grade, smoldering asymptomatic response. It may also arise due to the persistent infection by certain organisms such as tubercle bacilli or *Treponema pallidum*, prolonged exposure to highly toxic agents, either exogenous like silica or endogenous like plasma lipid component resulting into atherosclerosis, autoimmune like rheumatoid arthritis. Acute inflammation response is the initial response after the infection or trauma. It is non-specific in nature and

is the first line of defense of body after the danger<sup>8-9</sup>. In acute inflammation, there is increased level of copper, decreased level of zinc. There is occurrence of leukocytosis, thrombocytosis, negative nitrogen balance, increased BMR, increased lipogenesis and lipolysis. There is decrease in plasma protein level, increased C reactive protein level.

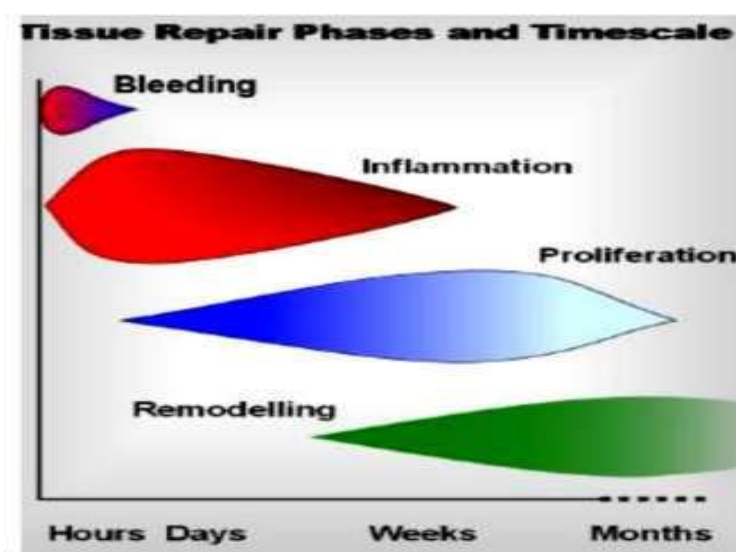


Figure 1.1: Tissue Repair phases and time scale

## **PLANT PROFILE-**

### **Zingiber officinale**

*Z. officinale* (Zingiberaceae) is an important plant with several ethnomedicinal and nutritional values therefore, used extensively worldwide as a spice, flavouring agent and herbal remedy. Traditionally, *Z. officinale* is used in Ayurveda, Siddha, Chinese, Arabian, Africans, Caribbean and many other medicinal systems to cure a variety of diseases viz, nausea, vomiting, asthma, cough, palpitation, inflammation, dyspepsia, loss of appetite, constipation, indigestion and pain. In last few decades, *Z. officinale* is extensively studied for its medicinal properties by advanced scientific techniques and a variety of bioactive compounds have been isolated from the different parts of the plant and were analysed pharmacologically. The plant is reported for antimicrobial activity, anticancer activity, antioxidant activity, antidiabetic activity, nephroprotective activity, hepatoprotective activity, larvicidal activity, analgesic activity, anti-inflammatory activity and immunomodulatory activity.

### **Taxonomical Classification**

- Kingdom: Plantae



- Division: Magnoliophyta
- Order: Zingiberales
- Family : Zingiberaceae
- Genus : Zingiber
- Species: *Z. officinale*

### **Panax ginseng**

*Panax ginseng*, ginseng also known as Asian ginseng, Chinese ginseng or Korean ginseng, is a species of plant whose root is the original source of ginseng. It is a perennial plant that grows in the mountains of East Asia<sup>108</sup>. *Panax ginseng* is primarily cultivated in Korea. While all South Korean ginseng is *Panax ginseng*, ginseng production in China encompasses both *Panax ginseng* and South China ginseng (*Panax notoginseng*).



Figure 5.2: *Panax ginseng*

### Scientific classification

- Kingdom: Plantae
- Clade: Angiosperms
- Clade: Eudicots
- Clade: Asterids
- Order: Apiales
- Family: Araliaceae
- Genus: *Panax*
- Species: *Panax ginseng*

## EXPERIMENTAL WORK-

### Collection of plant materials

Rhizome of *Zingiber officinale* and *Panax ginseng* were collected from local area of Bhopal in the period of February, 2025.



Figure 6.1: Collection of rhizome of *Zingiber officinale* and *Panax ginseng*

### Drying and storage of plant materials

Rhizome of *Zingiber officinale* and *Panax ginseng* were cleaned by tap water and a portion was dried at room temperature<sup>113</sup>. The dried samples were ground and passed through a sieve (20 meshes). The powdered drugs were kept in sealed containers and protected from light until used. Another portion of sample was used for maceration.

## Extraction by maceration process

Extraction of plant material refers to the process of isolating or extracting the active compounds or components from a plant material, such as leaves, flowers, roots, or bark, using solvents or other techniques. The extracted compounds can be used for various purposes, including pharmaceuticals, cosmetics, and food additives.

## Determination of percentage yield

The percentage yield is a measure of the efficiency of a chemical reaction. It compares the actual yield of a reaction to the theoretical yield, which is the amount of product that would be produced if the reaction were 100% efficient. The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

## Phytochemical analysis

Phytochemical analysis is the process of identifying and quantifying the bioactive compounds in plants, including fruits, vegetables, herbs, and spices. These compounds include various classes of compounds such as alkaloids, phenolics, flavonoids, terpenoids, steroids, and other secondary metabolites. The analysis of these compounds is important for

determining the nutritional and medicinal value of plants and their potential for various applications in food, pharmaceutical, and cosmetic industries. Phytochemical examinations were carried out on extracts as per the following standard methods.

**1. Detection of alkaloids:** Extracts dissolved individually in dilute Hydrochloric acid and filtered.

**a) Hager's Test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

**2. Detection of carbohydrates:** Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

**a) Fehling's Test:** Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

**3. Detection of glycosides:** Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

**a) Legal's Test:** Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Finding of pink to blood red colour indicates the presence of cardiac glycosides.

## 4. Detection of saponins

**a) Froth Test:** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of saponins.



## 5. Detection of phenols

**a) Ferric Chloride Test:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

### 6.7 In-vivo anti-inflammatory activity extract of *Zingiber officinale* and *Panax ginseng*

#### Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise- free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

#### Acute toxicity study

It was done according to Organization for Economic Co-operation and Development (OECD) guidelines 425 (up and down procedure). All the five rodents were administered 2000mg/kg of Hydroalcoholic extract of *Zingiber officinale* and *Panax ginseng* orally and observed continuously for a period of 14 days, every hourly for 24 hours, and every day for

14 days for its movements, grooming activity, exploring activity, writing reflex, eye movements, and convulsion etc [OECD guideline 2001]. The experimental dose of the extracts was selected as 100 and 200 mg/kg/p.o.

#### Experimental designs

Group	Treatment
Group 1	Carrageenan control (0.1 ml of 1% w/v)
Group 2	Carrageenan (0.1 ml of 1% w/v) + Indomethacin (10 mg/kg, p.o.)
Group 3	Carrageenan (0.1 ml of 1% w/v) + <i>Zingiber officinale</i> extract (100 mg/kg, p.o.)
Group 4	Carrageenan (0.1 ml of 1% w/v) + <i>Zingiber officinale</i> extract (200 mg/kg, p.o.)
Group 5	Carrageenan (0.1 ml of 1% w/v) + <i>Panax ginseng</i> extract (100 mg/kg, p.o.)
Group 6	Carrageenan (0.1 ml of 1% w/v) + <i>Panax ginseng</i> extract (200 mg/kg, p.o.)
Group 7	Carrageenan (0.1 ml of 1% w/v) + <i>Zingiber officinale</i> (100 mg/kg) + <i>Panax ginseng</i> (100 mg/kg)
Group 8	Carrageenan (0.1 ml of 1% w/v) + <i>Zingiber officinale</i> (200 mg/kg) + <i>Panax ginseng</i> (200 mg/kg)

## RESULTS AND DISCUSSION-

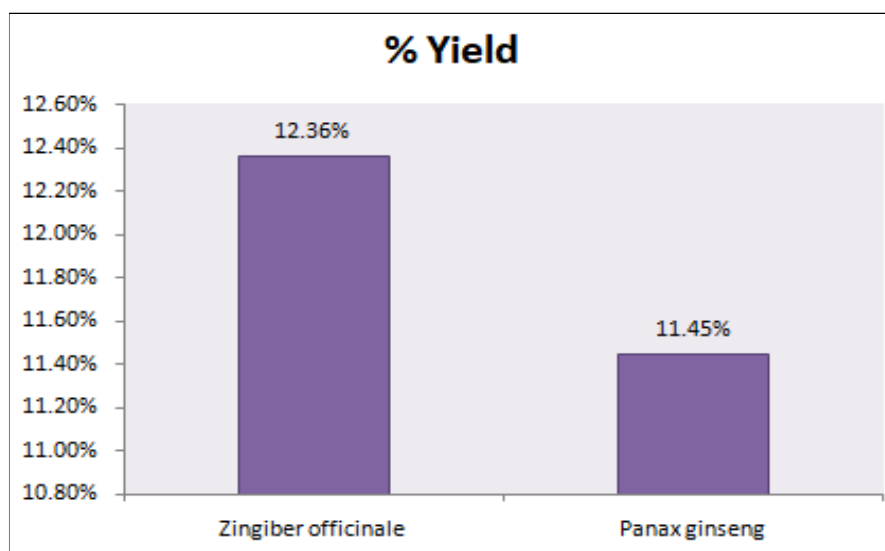
### Results of % yield of plant materials

The % yield of plant material can be influenced by various factors such as the quality and quantity of plant material used, the extraction method, and the solvents used for extraction. The % yield of plant material is expected to be low as plants contain a complex mixture of compounds that are not all soluble in the solvents used for extraction. The % yield of plant material is an important parameter that should be carefully monitored and reported in studies involving the extraction and isolation of bioactive compounds from plant sources.

#### % Yield obtained hydroalcoholic extract from *Zingiber officinale* and *Panax ginseng*

S. No.	Extract	Color	% Yield
1.	<i>Zingiber officinale</i>	Brown	12.36%
2.	<i>Panax ginseng</i>	Black	11.45%

The percentage yield of hydroalcoholic extracts obtained from *Zingiber officinale* and *Panax ginseng* is presented in Table 7.1. The extract of *Zingiber officinale* appeared brown in color and yielded 12.36%, while the extract of *Panax ginseng* was black and yielded 11.45%. The relatively higher yield from *Zingiber officinale* may be attributed to its richer content of polar phytoconstituents that are more readily soluble in hydroalcoholic solvents.



### 7.1 Results of phytochemical screening of *Zingiber officinale* and *Panax ginseng*

The preliminary phytochemical screening of the hydroalcoholic extract of *Zingiber officinale* revealed the presence of several important classes of bioactive compounds.

Table 7.2: Preliminary phytochemical screening of *Zingiber officinale*

S. No.	Phytoconstituents	Test Name	Hydroalcoholic extract
1	Alkaloids	Hager's Test	<b>Present</b>
2	Glycosides	Legal's Test	Absent
3	Carbohydrates	Fehling's Test	Absent
4	Flavonoids	Lead acetate	<b>Present</b>
5	Diterpenes	Copper acetate Test	Absent
6	Saponins	Froth Test	Absent
7	Proteins	Xanthoproteic Test	<b>Present</b>
8	Phenols	Ferric Chloride Test	<b>Present</b>

Table 7.6: Estimation of total phenolic and flavonoids content of *Zingiber officinale*

S. No	Extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1	Hydroalcoholic	0.42	0.76

Table 7.7: Estimation of total phenolic and flavonoids content of *Panax ginseng*

S. No	Extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1	Hydroalcoholic	0.34	0.57

## SUMMARY AND CONCLUSION-

Based on the experimental findings, the present study comprehensively evaluates the anti-inflammatory potential of hydroalcoholic extracts of *Zingiber officinale* and *Panax ginseng*. The extracts were prepared using hydroalcoholic solvent systems and exhibited notable yields 12.36% for *Zingiber officinale* (brown in color) and 11.45% for *Panax ginseng* (black in color). These yields suggest that the selected extraction method was effective in isolating bioactive constituents.



Preliminary phytochemical screening revealed the presence of key bioactive compounds in both extracts. *Zingiber officinale* extract tested positive for alkaloids, flavonoids, proteins, and phenols, while *Panax ginseng* extract contained carbohydrates, flavonoids, proteins, and phenols. Notably, both extracts contained flavonoids and phenolic compounds, which are well-known for their antioxidant and anti-inflammatory properties. The absence of glycosides, diterpenes, and saponins in both extracts further helps define their specific chemical profiles.

The estimation of total phenolic and flavonoid content was carried out using gallic acid and quercetin as standards, respectively. The results indicated that *Zingiber officinale* possessed higher levels of phenols (0.42 mg/100 mg) and flavonoids (0.76 mg/100 mg) as compared to *Panax ginseng*, which showed 0.34 mg/100 mg of phenols and 0.57 mg/100 mg of flavonoids. These values reinforce the expectation that *Zingiber officinale* may exert stronger biological activity, particularly in inflammatory conditions.

The in vivo anti-inflammatory activity of the extracts was evaluated using the carrageenan- induced paw edema model in Wistar rats. In the control group, a progressive increase in paw volume was observed, peaking at 4 hours post-carrageenan administration. The standard drug, Indomethacin (10 mg/kg), significantly reduced paw edema throughout the observation period. Both *Zingiber officinale* and *Panax ginseng*, when administered individually at 200 mg/kg, showed a significant reduction in paw swelling from 2 hours onwards, with *Zingiber officinale* slightly outperforming *Panax ginseng*. However, at 100 mg/kg, the reduction was moderate, indicating a dose-dependent response.

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