# Standardization, Chemical and Pharmacological Evaluation of Soymida Febrifuga for Antimicrobial and Anti-Inflammatory Activities

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## ABSTRACT

*Soymida Febrifuga* is a medicinal plant traditionally employed in the treatment of infectious and inflammatory disorders. Despite its ethnopharmacological relevance, systematic evaluation of its phytochemical and pharmacological properties is limited. This study aimed to investigate the phytochemical composition, antibacterial, antifungal, and anti-inflammatory activities of *Soymida Febrifuga* bark extracts. Bark samples were collected, authenticated, and extracted using methanol and ethyl acetate via Soxhlet extraction. Qualitative phytochemical screening was performed using standard assays, while quantitative estimation of phenolics and flavonoids was conducted using gallic acid and quercetin calibration curves. Antimicrobial activity was assessed by disc diffusion and agar-well diffusion methods against Gram-positive and Gram-negative bacterial strains and fungal species. Anti-inflammatory activity was evaluated in BALB/c mice using carrageenan-induced paw edema, with indomethacin as the reference drug.

Phytochemical screening confirmed the presence of carbohydrates, glycosides, phenols, tannins, flavonoids, diterpenes, steroids, proteins, and amino acids. Quantitative analysis revealed high phenolic (118.45 mg GA/g) and flavonoid (34.42 mg QE/g) content in the ethyl acetate fraction. Methanolic extracts exhibited strong antibacterial activity, particularly against Proteus vulgaris (29 mm), Staphylococcus aureus (27 mm), and Pseudomonas aeruginosa (26 mm), while ethyl acetate extracts showed moderate activity. No antifungal activity was observed. Anti-inflammatory evaluation demonstrated dose-dependent inhibition of paw edema, with the highest dose (400 mg/kg) achieving 47.98% inhibition at 5 hours, compared to 69.31% with indomethacin. *Soymida Febrifuga* bark extracts contain bioactive phytochemicals, notably phenolics and flavonoids, which contribute to significant antibacterial and anti-inflammatory activities. These findings validate its traditional use and highlight its potential as a source of therapeutic agents for infectious and inflammatory diseases.

**Keywords:** Soymida febrifuga; gallic acid; quercetin; antibacterial activity; antifungal evaluation; anti-inflammatory activity

## INTRODUCTION

Plants use like nutritive, therapeutic and bio-pesticidal are developing a common trend in different parts of the world. The tendency towards plants as a medicinal agent around the globe is due to lesser side effects, better compatibility with human body, relatively reasonable cost and well efficiency as compared to synthetic drugs [1]. World Health Organization assessed that 4 billion population use herbal medicine for their primary health care. According to the WHO survey that traditional healers treat 90 % patients in Bangladesh, 80% in India, 75% in Nepal, and 65% in Sri Lanka. Plants have been rich sources of medicines since long as they produce a

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multitude of biologically-active compounds, most of which possibly uses in the treatment of different diseases in human and animals [2]. Plants have always acted as a major part in the handling of human and animal diseases. It was generally believed in the early nineteen century that utilization of plants as medicines has involved the isolation of morphine from opium. Exploration of plants for drug discovery led to the isolation of initial drugs such as morphine, quinine, cocaine, and digitoxin. In the year 2001 and 2002, about one fourth of the best-selling drugs worldwide were isolated from natural products or their derivatives [3].

Antibiotics, which can destroy the natural defenses of the body, such as the innate immunity, are either fatal or chemo-preventive to microbes. Sometimes they are acting by inhibiting the development, by means of a membrane disorganizing molecule, of protein, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or of other particular performance from time to time [4]. Antibiotics can also enter bacteria's cell walls by attaching to them via energy-dependent transport pathways in ribosomal locations, inhibiting protein synthesis. The world's largest concern to public health is antifungal resistance (AMR), which threatens the capacity to prevent and cure a range of infectious diseases [5].

Despite being a worldwide problem, the distribution of AMR amongst nations has been divergent and has a greater impact on developing countries. This higher burden for impoverished nations might lead to limited access to new antibiotics as well as an increase in financial burdens and the failure, which may result in poorer treatment, to pay for second-line antibiotics [6].

Antibiotics have definitely been a boon to human civilization in combating diseases or microorganisms, saving millions of lives. Misuse of antibiotics in bacteria, which are isolates from food-producing animals, was linked to significant resistance rates in the use of important antimicrobials in human health [7]. While antimicrobial resistance emerges and spreads naturally, there is an enhanced selection pressure, which is mostly generated by the overuse and abuse of antibiotics in both human and animal medicine.

Different authorities have undertaken a huge number of new efforts to address this problem in the past decade [8]. These are India Clen (Indian Clinical Epidemiology Network). The NGO consortium launched in March 2008 by IIMAR (Indian Initiative for Management of Antibiotic Resistance) with support from WHO to promote prudently using antimicrobials, generated some quality AMR data for Pneumococcal *H. Influenzae* in the whole country.

Infectious diseases remain among the leading causes of morbidity and mortality worldwide, despite remarkable advances in medicine and public health [9-12]. According to the World Health Organization (WHO), lower respiratory tract infections, diarrheal diseases, and tuberculosis continue to account for millions of deaths annually, particularly in low- and middle-income countries. The emergence of novel pathogens, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has further underscored the vulnerability of global populations to infectious threats [13].

The medicinal plant *Soymida Febrifuga (Roxb.)* A. Juss., belonging to the family *Meliaceae*, has been traditionally employed in Indian ethnomedicine for the treatment of fever, gastrointestinal disorders, and skin ailments. Its bark, leaves, and seeds are reported to contain bioactive compounds such as alkaloids, flavonoids, tannins, and saponins, which are associated with antimicrobial and anti-inflammatory activities [14].

Despite its long history of use, systematic pharmacological validation and mechanistic studies remain limited. This research is envisaged to bridge the gap between traditional knowledge and modern scientific evidence, with a focus on antimicrobial and anti-inflammatory potential. Given the global burden of infectious and inflammatory diseases, and the growing interest in plant-based therapeutics, *S. Febrifuga* represents a promising candidate for further exploration [15].

This research is envisaged to bridge the gap between ethnomedicine and modern pharmacology. By systematically investigating *Soymida Febrifuga*, the study aims to provide credible scientific data that can support its integration into evidence-based medicine. The dual focus on antimicrobial and anti-inflammatory activity aligns with current global health priorities, offering potential solutions to two of the most pressing challenges in biomedical science: infectious disease management and chronic inflammation [16].

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# MATERIALS AND METHODS

## Material

Analytical balance, Hot air oven, Autoclave, Laminar air flow, Incubator, Micropipettes. They were carried out on Mueller-Hinton agar (MHA) for bacteria, and Sabouraud Dextrose Agar (SDA) for fungus, using the agar-well diffusion method for crude extracts and the conventional disc diffusion technique for the crude extracts of chosen organic solvents.

## Preparation of Plant for Extract

The bark of Soymida Febrifuga was collected from forest, Sagar District, M.P., India and authenticated through botanical authentication carried out by a qualified botanist at the Department of Botany, Safia College, Bhopal, MP. In order to make a fine powder, the raw materials of medicinal plants were thoroughly washed with running tap water followed by distilled water multiple times and then air dried in the shade for a couple of days. A 40% (w/v) ethyl acetate and methanol extraction was carried out on the crude powdered plants using the Soxhlet method for 6-8 hours and then filtered. They were stored in a refrigerator at 4°C until they were needed [17].

## Phytochemical Screening:

Standard qualitative techniques were used to assess the phytochemical composition of the extracts for a variety of phytoconstituents. Extraction was carried out to determine if the extracts included physiologically active chemicals such as alkaloids and glycosides; amino acids; proteins; steroids; and diterpenes; flavonoids; phenolics; tannins, etc.

### [a] Detection of Tannins:

They were cooked in a test tube for 10 minutes with 20 mL of water to extract the extracts (4 mL). Some ferric chloride (5%) was added, and after 10 minutes, a brownish green or blue-black color was noticed [18].

### [b] Detection of Saponins:

10ml of the extract was mixed and heated till boiling. Saponins can be detected by frothing (the appearance of a creamy mass of tiny bubbles) [19].

### [c] Detection of Alkaloids:

On a steam bath, 10 mL of 1 percent aqueous hydrochloric acid was mixed with 4 mL of each extract for 10 minutes. Mayer's reagent was added to 1 mL of the extract. Presumably, alkaloids were present when precipitation occurred with these reagents [20].

### [d] Detection of Flavonoids:

There were few drops of sodium hydroxide in the extract (5 mL). In the presence of flavonoids, a strong yellow hue appears, which fades away when diluted acid is introduced [21].

### [e] Detection of Phenols

Two to three drops of ferric chloride solution were added to the extracts (5 mL). The presence of phenols is indicated by the formation of a blue-black hue [22].

### [f] Detection of Glycosides:

With sodium nitroprusside-pyridine-sodium hydroxide, the extract (5 mL) was processed. It is believed that cardiac glycosides are responsible for the formation of pink to blood red hue [23].

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue I January 2026

**[g] Detection of Carbohydrates**

The extracts (5 mL) were treated with a few drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates [24].

**[h] Detection of Diterpenes**

The extracts (5 mL) were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes [25].

**[i] Detection of steroids**

The extracts (5 mL) were dissolved in 10 mL of chloroform. A few drops of concentrated sulphuric acid were carefully added to form a lower layer. A reddish color formed at the interphase indicates the presence of a steroid ring [26].

**[j] Detection of proteins and amino acids**

The extracts (5 mL) were added 0.25% w/v ninhydrin reagent and boiled for few minutes. Formation of blue color indicates the presence of amino acid [27].

**Pharmacological Evaluation**

**Test organisms**

Four-gram negative bacterial strains (Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa and Klebsiella pneumonia.) and one-gram positive bacterial strains (Staphylococcus saprophyticus) from standard cultures were used as test strains.

**Determination of antibacterial activity by disc diffusion method**

The aqueous and ethanolic crude extracts of plants were screened for antibacterial activity by the paper disc diffusion method. From the 50 mg/mL typical solution of each extract of plants, 40 µL aliquots were transferred onto blank sterile paper discs (6 mm diameter). The dried discs were carefully and firmly placed on nutrient agar medium (UK) previously inoculated with a bacterial suspension and incubated at 37ºC for 24 hr. The appropriate solvent was used as control to determine the sensitivity of the tested strains. After incubation, plates were examined for the presence of zones of growth inhibition, and the diameters of these zones were measured in mm. Tests were performed in duplicate under sterile conditions [28-30].

**Anti-bacterial and Anti-fungal testing of the crude extracts**

The antibacterial and antifungal activity of crude extracts was evaluated. Each butt should be prepared with 150 ml Mueller Hinton agar, 20 ml of medium, and 0.02 ml of a culture of various species. In order to build wells, cork borers were employed after the corks had hardened and dried. Fill this well with 0.1ml of your organic solvent bark extract working solution, which you've selected. For 24 hours, they are kept at 37°C. A measurement of the inhibitory zone was made after the incubation time [31].

**Anti-inflammatory activity**

BALB/c mice were used for investigation of *Soymida Febrifuga* extract for their possible anti-inflammatory effect using standard method [32]. In this experiment, 5 groups of animals were used, containing six animals in each group. Control (normal saline, Group-I), Test groups (II-IV), received their respective doses, and group V received Indomethacin (10 mg/kg s.c). After 30 minutes of treatment, carrageenan (0.05 ml of 1 % solution) was injected subcutaneously to the right hind paw of each mouse. Digital plethysmometer using to measure the paw volume at 1-5 hours. Percentage of inhibition of edema was determined through comparison of the average paw swelling in sample treated and standard treated groups with that of control [33]. The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue I January 2026

the requirements of National guidelines of CCSEA. IAEC Approval No. VEDIC/CCSEA/IAEC/18, Dated 11/04/2025).

### Statistical analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD). Group mean values were compared using two-way analysis of variance (ANOVA) to determine statistical differences among treatments. Statistical analyses were conducted using GraphPad Prism software (version 8.4). A p-value of $< 0.001$ was considered statistically significant.

## RESULTS AND DISCUSSION

### Phytochemical Screening:

The bark extract of *Soymida Febrifuga* was used for the phytochemical constituent estimation. The *Soymida Frbrifuga* was used for the qualitative test and the data was represented in Table 1. The Plant extract shown the sign of the flavonoid and phenolic content and stated that gallic acid and quercetin content.

**Table 1: Soymida Febrifuga bark extract phytochemical analyses**

| Name of the Compound | Name of the Test | Result |
|---|---|---|
| Carbohydrates | Molisch test | + |
| Glycosides | Legal test | ++ |
| Phenols | Ferric chloride test | ++ |
| Tannins | 5% ferric chloride | ++ |
| Flavonoids | Alkaline reagent test | ++ |
| Diterpenes | Copper acetate test | + |
| Steroids | Chloroform + acetic acid + $H_2SO_4$ | + |

"+" = present; "++" = more quality

### Quantitative analysis of phytochemicals

The total phenols and flavonoid compounds in crude extracts and different solvent fractions of Soymida Febrifuga are presented in Table 2 and Figure 1. The phenolic compounds among the various extracts and fractions were expressed in terms of gallic acid (GA) using standard curve equation, Abs = 0.0065[GA] + 0.0181, $R^2$ = 0.9915. The phenolic content was reported higher in crude extract (82.80 mg GA/g) and ethyl acetate fraction (118.45 mg GA /g) followed by DCM fraction (68.82 GA /g) of *Soymida Febrifuga*.

**Table 2: Absorbance of standard compound (Gallic acids) at $\lambda_{max}$**

| S. No. | Gallic acids (mg/ml) | Absorbance (mean value) |
|---|---|---|
| | 2 | 0.017 |
| | 4 | 0.026 |
| | 6 | 0.044 |
| | 8 | 0.052 |
| | 10 | 0.066 |

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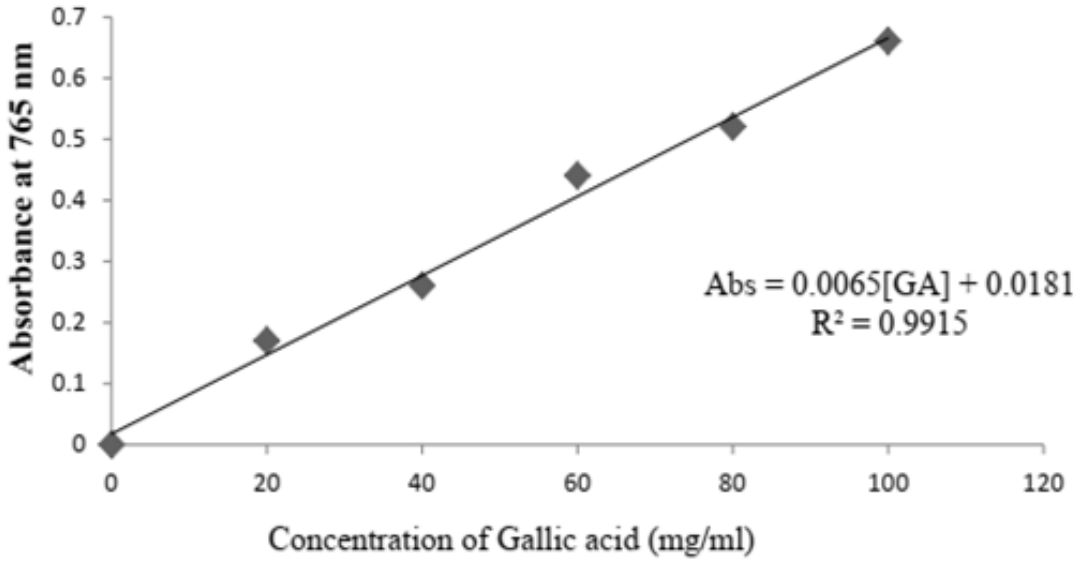


**Figure 1: Calibration Curve of Gallic acid**

The flavonoids content of extracts and different fractions were expressed in terms of quercetin (QE) using standard curve equation, Abs = 0.0042[QE] + 0.0138, R2 = 0.9829 (Table 3 & Figure 2). The flavonoid content was also found higher in crude extract (27.72 mg QE/g) and ethyl acetate fraction (34.42 mg QE/g) of *Soymida Febrifuga* (Table 4).

**Table 3: Absorbance of standard compound (Quercetin) at $\lambda_{max}$ = 415 nm**

| S. No. | Concentration (mg/ml) | Absorbance (mean value) |
|---|---|---|
| 1. | 2 | 0.012 |
| 2. | 4 | 0.019 |
| 3. | 6 | 0.025 |
| 4. | 8 | 0.032 |
| 5. | 10 | 0.045 |

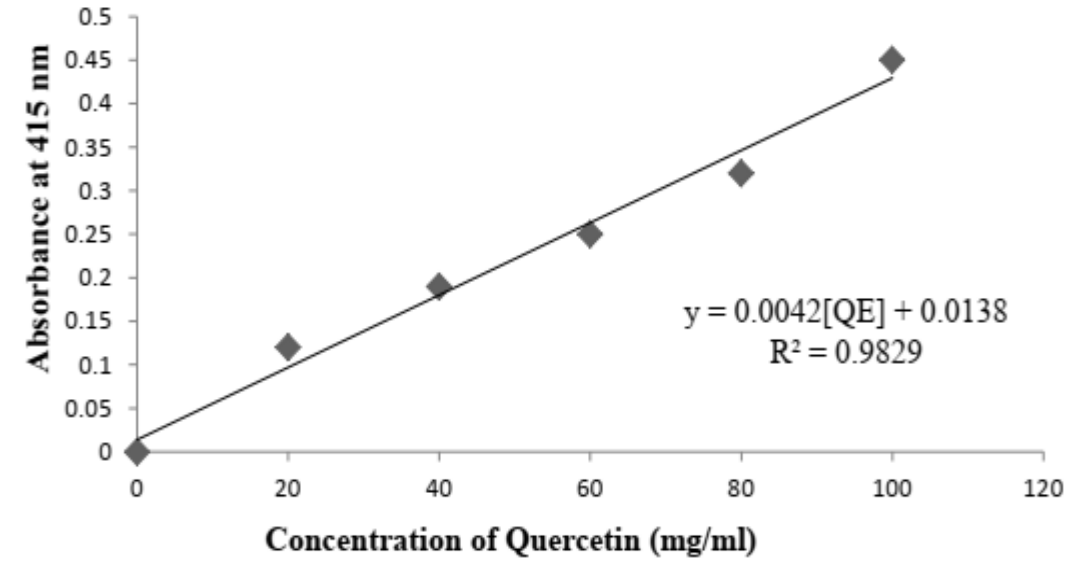


**Figure 2: Calibration curve of Quercetin**

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue I January 2026

**Table 4: Total phenolic and flavonoids compounds of crude extracts and fractions of Soymida Febrifuga**

| Extracts & Fractions | Total Phenolic (mg of Gallic acid/g of sample) | Total Flavonoid (mg of Quercetin /g of sample) |
|---|---|---|
| Methanolic extract | 82.80 ± 0.08 | 27.72 ± 0.06 |
| Hexane fraction | 8.23 ± 0.08 | 14.42 ± 0.41 |
| Dichloromethane fraction | 68.82 ± 0.07 | 18.55 ± 0.07 |
| Ethyl acetate fractions | 118.45 ± 0.08 | 34.42 ± 0.06 |

All values are expressed as mean ± standard deviation (n = 3).

**Anti-bacterial and anti-fungal testing**

**Antibacterial activity of Soymida Febrifuga:**

The methanolic extract of *Soymida Febrifuga* confirmed exact antibacterial interest towards S. aureus, P. Aeruginosa and P. Vulgaris this is 27 mm, 26 mm, 29 mm respectively, mild interest towards K. pneumonia and S. dysentriae this is 23 mm and 22 mm respectively and moderate interest towards E. coli and S. typhi this is 19 mm and 19 mm respectively.

The ethyl acetate extract of *Soymida Febrifuga* confirmed top antibacterial pastime towards P. vulgaris and P. aeruginosa this is 22 mm and 21 mm respectively (Table 5), mild pastime towards S. aureus this is 20 mm and slight pastime towards E. coli, K. pneumonia, S. typhi and S. dysentriae this is 15 mm, 12 mm, 13 mm and 16 mm respectively (Figure 3).

**Table 5: Anti-microbial activity of different crude bark extracts against Bacterial and Fungal species**

| Microbial species | Zone of Inhibition (mm) | |
|---|---|---|
| | Methanolic Extract | Ethyl Acetate Extract |
| Staphylococcus aureus | 27 mm | 20 mm |
| Klebsiella pneumonia | 23 mm | 12 mm |
| Pseudomonas aeruginosa | 26 mm | 21 mm |
| Salmonella typhi | 19 mm | 13 mm |
| Escherichia coli | 19 mm | 15 mm |
| Shigella dysentriae | 22 mm | 16 mm |
| Proteus vulgaris | 29 mm | 22 mm |

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue I January 2026

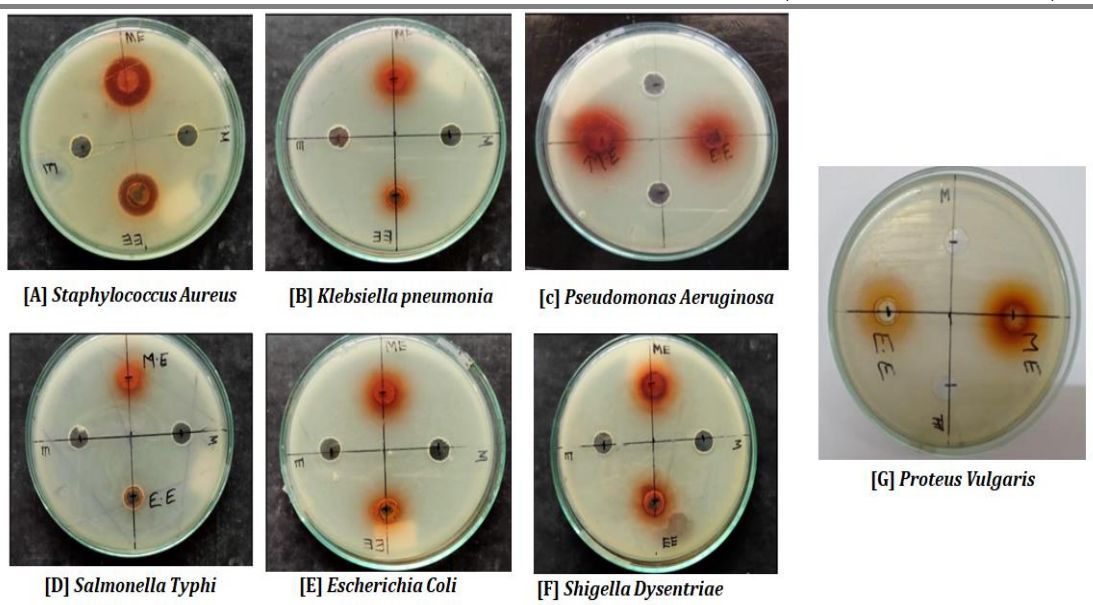


**Figure 3: Anti-microbial activity of different crude bark extracts against Bacterial and Fungal species**

**Anti-inflammatory activity**

Soymida Febrifuga extract showed significant anti-inflammatory effect when compared to control and results are summarized in Table 6 and 7. Crude extract (400 mg/kg) showed significant anti-inflammatory effect ($p<0.01$) after 3 hrs. of carrageenan administration and this condition remained significant till 5 hours.

**Table 6: Anti-inflammatory activity of crude extract**

| **Sample** | **Doses (mg/kg)** | **Increase in paw edema size (mm) ± S.E.M** | |
|---|---|---|---|
| | | **3rd hour** | **5th hour** |
| Saline | - | 5.73 ± 1.42 | 6.94 ± 1.67 |
| Extract | 100 | 5.54 ± 1.26 | 6.01 ± 1.43 |
| | 200 | 4.65 ± 0.73* | 4.82±0.88* |
| | 400 | 3.58±0.47** | 3.61±0.52** |
| Indomethacin | 10 | 3.05±0.69** | 2.13±0.34** |

Mean ± Standard error of mean (n = 6). Analysis of variance (ANOVA) followed by Dunnett‟s test was applied to determine statistical significance.*P< 0.05, **P< 0.01.

**Table 7: Percent inhibition edema of the crude extract of *Soymida Febrifuga***

| **Sample** | **Doses (mg/kg)** | **Percentage of edema Inhibition** | |
|---|---|---|---|
| | | **3rd hour** | **5th hour** |
| Saline | - | - | - |
| Extract | 100 | 3.31 | 13.40 |
| | 200 | 18.85 | 30.55 |

| | | | |
|---|---|---|---|
| | 400 | 37.52 | 47.98 |
| Indomethacin | 10 | 46.77 | 69.31 |

Similarly, the same dose also showed maximum percent inhibition (47.98 %) of paw edema at 5 hrs. of observation. The percent inhibitory effect of indomethacin (69.31%) was greater than that of the highest dose of our test extract. The anti-inflammatory effect of extract was in dose-dependent manner.

## CONCLUSION

Carbohydrates, glycosides, phenols, tannins, flavonoids, proteins and amino acids, diterpenes, and steroids were found in the preliminary qualitative phytochemical study of *Soymida Febrifuga*, which might be useful in the treatment of infectious illnesses. The *Soymida Febrifuga* contains the flavonoid and phenolic content as Gallic acid and Quercetin. Therefore, *Soymida Febrifuga* bark extracts have a broad spectrum of action against a panel of bacteria responsible for the most prevalent bacterial illnesses, while exhibiting no activity against tested fungus species. It was determined that alcoholic bark extract has a good anti-inflammatory action by testing the anti-inflammatory activity of plants.

**Compliance with ethical standards:** Animal (BALB/c mice) used for the study. IAEC Approval No. VEDIC/CCSEA/IAEC/18, Dated 11/04/2025).

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